

Silage Inoculant and Its Role in Enhancing Fermentation and Aerobic Stability Across Multiple Crops

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Introduction

Silage inoculants are often developed for specific forage types due to fermentation dynamics influenced by crop conditions. Farmers frequently face challenging conditions during ensiling, such as delays due to contractor availability or adverse weather conditions, e.g., flooding or drought (Jatkauskas *et al.*, 2024). These challenges can prevent the achievement of optimal dry matter (DM) levels for ensiling, which are crucial for establishing the ideal environment for microbial fermentation. Silage inoculants can enhance the fermentation process by directing it through specific metabolic pathways to produce targeted acids during fermentation (Muck *et al.*, 2018). The objective of this study was to assess the effects of using a combination of *Lactococcus lactis* and *Lentilactobacillus buchneri*, on the fermentation process and aerobic stability of grass, alfalfa, and corn silages.

Materials and methods

The study was conducted on grass, alfalfa, and corn forages harvested at different times. Each crop was prepared and ensiled in 3-liter mini-silos as follows: grass was wilted to 29.4% DM and chopped to a length of 2–3 cm before ensiling. Alfalfa was wilted to 29.7% DM and chopped similarly to grass. Whole-crop corn (34.0% DM) was chopped with a forage harvester set to a 10 mm chop length. Ten mini-silos per treatment (five for fermentation quality and five for aerobic stability testing) were prepared. The laboratory study included two treatments for each forage type: (1) control (C), with no inoculant applied, and (2) microbial-based inoculant (*Lactococcus lactis* (DSM11037) and *Lentilactobacillus buchneri* (DSM22501); SILOSOLVE® FC, Novonesis, Lyngby, Denmark) applied at 150,000 CFU/g of fresh matter. Silages were stored at room temperature ($20.0 \pm 1^\circ\text{C}$) for 90 days. At day 90 of storage, samples of the ensiled crops (five replicates for each treatment) were analyzed for DM, fermentation parameters (including pH, ethanol, 1,2-propanediol, lactic acid, and volatile fatty acids [VFAs]), and microbial characteristics (LAB, yeast, mold, and Clostridia spp.). DM content was corrected for the loss of volatile compounds during oven drying (Weissbach and Strubelt, 2008). The silages were subsequently exposed to air by transferring them into open polystyrene boxes and storing them under controlled ambient conditions for an aerobic challenge lasting 21, 23, and 27 days for corn, grass, and alfalfa silages, respectively. Aerobic stability was defined as the duration (in hours) for which the silage remained stable, i.e., before the temperature rose 3°C or more above ambient temperature. Each mini-silo served as the experimental unit for statistical analysis, which was conducted using the PROC GLM procedure in SAS 9.4 software. The model included treatment as the main fixed effect for each inoculant, with statistical significance defined at $P \leq 0.05$. Statistical analysis was performed separately for each forage type (grass, alfalfa, and corn) to account for crop-specific fermentation dynamics.

Results

In all three crops, the inoculation with *Lactococcus lactis* and *Lentilactobacillus buchneri* led to consistent improvements in fermentation and aerobic stability. For grass silage, dry matter (DM) increased from 27.3% to 28.2% ($P \leq 0.001$), and in corn, it rose from 32.0% to 33.0% ($P \leq 0.001$), while no significant difference was observed in alfalfa. The pH remained stable across all crops. Lactic acid concentrations increased slightly in alfalfa (5.6% to 6.3%, $P \leq 0.05$) and corn (3.9% to 4.6%, $P \leq 0.05$), reflecting the contribution of homofermentative bacteria in boosting lactic acid production while reducing butyric acid (e.g., 0.09% to 0.02% in alfalfa, $P \leq 0.001$). Ammonia-N levels decreased significantly in all silages, with reductions of 20 g/kg N in corn ($P \leq 0.001$).

Acetic acid concentrations increased significantly due to *L. buchneri* activity, reaching 5.1% in grass ($P \leq 0.001$), 4.5% in alfalfa ($P \leq 0.001$), and 2.5% in corn. Additionally, this increase contributed to a reduction in ethanol concentrations (e.g., 0.8% to 0.6% in grass, $P \leq 0.001$) and supported longer aerobic stability times, which increased from 124.8 to 526.8 hours in grass ($P \leq 0.001$), from 536.4 to 639.6 hours in alfalfa ($P \leq 0.05$), and from 93.6 to 199.2 hours in corn ($P \leq 0.05$).

Table 1. Fermentation characteristics and aerobic stability of grass, alfalfa, and corn silages

	Grass		Alfalfa		Corn		SEM
	C	FC	C	FC	C	FC	
Dry matter (%)	27.33	28.16***	27.76	28.22	31.97	32.95***	0.02
pH	4.36	4.37	4.88	4.84	3.84	3.80	0.01
Lactic acid (%)	6.68	6.55	5.58	6.32*	3.87	4.60*	1.38
Acetic acid (%)	2.82	5.14***	2.47	4.52***	2.08	2.51	0.13
Butyric acid (%)	0.06	0.04	0.09	0.02***	0.05	0.03*	0.01
Propionic acid (%)	0.04	0.11***	0.04	0.25***	0.03	0.02	0.01
Ethanol (%)	0.75	0.60***	1.10	0.87***	0.80	0.65***	0.02
1,2 Propandiol (g kg /DM)	0.28	1.51***	0.39	2.46***	0.24	0.52**	0.07
Ammonia N (g/kg N)	56.74	45.33**	69.72	53.77**	64.64	44.56***	0.18
LAB (Log10 CFU/g)	7.58	8.41***	6.41	7.12***	7.53	8.25***	0.11
Clostridia (Log10 CFU/g)	1.65	1.26**	2.03	1.79	1.57	1.20	0.06
Yeast (Log10 CFU/g)	1.84	1.12***	1.97	1.12***	2.16	1.24***	0.06
Moulds (Log10 CFU/g)	1.42	1.12	1.86	1.06***	2.11	1.40***	0.01
Fermentation DM losses (%)	8.10	5.19***	7.79	6.16***	7.05	4.10***	0.22
Aerobic stability (hours)	124.80	526.80***	536.40	639.60*	93.60	199.20*	21.16

C – non-inoculated; FC – inoculated with *Lactococcus lactis* and *Lentilactobacillus buchneri*; *, **, *** – indicate significant differences between control and inoculated treatments at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

LAB populations increased across treatments (e.g., 8.41 vs. 7.58 log CFU/g in grass, $P \leq 0.001$), while yeast and moulds were reduced (e.g., yeast decreased from 2.16 to 1.24 log CFU/g in corn, $P \leq 0.001$). The oxygen-scavenging effect of *L. lactis* during early fermentation limited the growth of spoilage microorganisms and established a favourable microbial balance. Dry matter losses during fermentation were significantly lower for all inoculated treatments ($P \leq 0.001$).

Conclusions

The combination of *Lactococcus lactis* (DSM11037) and *Lentilactobacillus buchneri* (DSM22501) on grass, alfalfa, and corn silages improved fermentation quality and aerobic stability across all crops, significantly reducing DM losses and suppressing undesirable microbes, demonstrating the versatility of the inoculant across crops.

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Effects of Oxygen Barriers on Losses, Fermentation and Microbial Counts of Sorghum Silage

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Keywords: mold counts, silage hygiene, silage covers, yeast counts.

Introduction

The preservation of silage quality is critical for ensuring consistent and high nutritional value in livestock feed. Maintaining anaerobic conditions during the ensiling process is paramount to minimizing nutrient losses and ensuring high feed quality. The type of cover used to seal silos plays a pivotal role in this process. Conventional polyethylene (PE) covers are commonly employed due to their availability and low cost. However, their relatively high oxygen permeability can compromise silage quality by promoting aerobic spoilage, which leads to dry matter (DM) losses and reduced nutrient preservation. Oxygen barrier films (OB), have demonstrated the potential to minimize oxygen penetration, thereby improving fermentation efficiency and extending aerobic stability (Borreani et al., 2018). These films are designed to significantly reduce oxygen transmission rates, creating an environment more conducive to optimal fermentation (Wilkinson & Fenlon, 2014). Limited studies have explored the efficacy of these covers in sorghum silage, a crop with distinct fermentation dynamics due to its high fiber and sugar concentrations. This study aimed to compare the performance of a conventional PE cover and two OB in preserving sorghum silage for a short storage period, focusing on their effects on fermentation profile, hygiene and DM losses.

Material and Methods

Fresh whole-plant sorghum was harvested from an experimental field (Citra, FL) in late October of 2024. Forage was chopped at a theoretical length of cut of 15 mm. Two samples were taken and analyzed for nutritive value in a commercial laboratory using NIR (DM, CP, sugar, NDF, and ash; $29.0 \pm 0.94\%$, $7.73 \pm 0.07\%$ DM, $10.7 \pm 0.44\%$ DM, $49.4 \pm 1.74\%$ DM, and $4.91 \pm 0.15\%$ DM; mean $\pm$ SD, respectively). Harvested forage (580 ± 12.3 g) was used to fill Ball® mason jars (used as experimental silos; vol. 946 mL) yielding an average density of 613 ± 12.9 kg/m³. Packed forage was covered with the following treatments in four replications; PE (Control; standard polyethylene film, 150 μ m), OB1 (45 μ m, oxygen transmission rate; OTR < 0.4 cm³/m²/24 h; DIN 53380-3), and OB2 (40 μ m, OTR < 20 cm³/m²/24 h; DIN 53380-3), and thoroughly sealed with elastic bands to avoid air entry from the sides. Experimental silos were allowed to ferment in room temperature for 52 days and weighed before opening for gas loss estimation and DM recovery. Inedible silage was characterized visually by the presence of moldy, darker, slimy silage on the top layer closest to plastic covers. Inedible silage was removed, weighed and discarded. The remaining silage was homogenized, and samples were taken, for chemical analysis (NIR), yeast and mold counts (AOAC Official Method, 2014.05) and fermentation profile (Muck and Dickerson, 1988). Estimated aerobic stability was calculated using the log of yeast counts and the equation described by Kung et al. (1998). The experiment followed a completely randomized design. Data were checked for normality using the UNIVARIATE procedure of SAS (SAS Studio version 9.4; SAS Institute) and analyzed using the GLM procedure (SAS Studio version 9.4; SAS Institute). Significance was declared at $P \leq 0.05$, and a tendency was declared at $0.05 < P \leq 0.10$.

Results

Treatment means related to silage losses, microbial counts and aerobic stability are reported on Table 1. Gas loss was greatest for PE when compared with the oxygen barrier treatments ($P < 0.01$), while DM recovery was greatest for OB1 and OB2 ($P < 0.01$). Inedible silage was greatest for PE and represented 3.3 and 2.2 times more inedible silage when compared with OB1 and OB2, respectively ($P < 0.01$). The greatest loss is probably related to the greater entry of air in the PE covered silos and consequent greater proliferation of detrimental aerobic microorganisms such as yeast and molds that were 2 log greater for PE when compared to OB1 and OB2 ($P < 0.01$), due to greater yeast counts in the PE treatment, estimated aerobic stability was also greater for this treatment when compared to the OB treatments ($P = 0.04$). Fermentation profile means are reported on Table 2. pH was greatest for PE covered silos ($P = 0.02$), but no effect of treatments was observed for lactate, acetate, and ammonia concentrations ($P > 0.14$). However, ethanol concentration was lower for PE when compared with OB treatments ($P = 0.03$). Silage DM was greater for OB treatments, while concentrations of fiber components (NDF, ADF and lignin) were lower ($P < 0.01$, data not shown).

Conclusion

In this study we demonstrated that the use of OB with OTR < 20 cm³/m²/24 h as silage covers in a short storage period are effective at improving silage hygiene and reducing DM losses and inedible sorghum silage when compared with PE. In addition, OB with OTR < 0.4 cm³/m²/24 h significantly reduced inedible sorghum silage to a greater extent compared to all other treatments.

Table 1. Effects of silage covers with different oxygen transmission rates on gas loss, DM recovery, inedible silage, yeast and mold counts and estimated aerobic stability of sorghum silages fermented for 52 days.

Item	Treatments			SEM	P-value
	PE	OB1	OB2		
Gas loss, %	8.05 ^a	4.43 ^b	4.33 ^b	0.25	<0.01
DM recovery, %	86.0 ^b	90.1 ^a	91.4 ^a	0.45	<0.01
Inedible, %	24.6 ^a	7.40 ^c	11.1 ^b	1.50	<0.01
Yeast, log(cfu)/g	6.39 ^a	4.42 ^b	4.73 ^b	0.20	<0.01
Mold, log(cfu)/g	5.11	ND [§]	ND	0.55	<0.01
Estimated aerobic stability, h	23.5 ^a	113.5 ^b	99.1 ^b	9.50	0.04

Means within a row not followed by the same letter are different ($P < 0.05$).

[§]ND= not detected; limit of detection 1000 cfu/g.

Table 2. Effects of silage covers with different oxygen transmission rates on fermentative profile of sorghum silages fermented for 52 days.

Item	Treatments			SEM	P-value
	PE	OB1	OB2		
pH	3.70 ^a	3.67 ^b	3.64 ^c	0.01	0.02
Lactate, % of DM	5.12	5.57	5.28	0.11	0.14
Acetate, % of DM	1.30	1.32	1.30	0.08	0.96
Ethanol, % of DM	1.78 ^a	2.18 ^b	2.10 ^b	0.10	0.03
Ammonia, % of DM	2.30	2.25	2.23	0.05	0.81

Means within a row not followed by the same letter are different ($P < 0.05$).

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Spent Mushroom Substrate and Oilseeds Influenced the Nutritive Quality and Greenhouse Gas Emissions of Corn Silage

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The increasing cost of conventional feed ingredients and the urgent need for environmentally sustainable feeding strategies necessitate the exploration of alternative feed resources. The integration of oilseeds (OS) with spent mushroom substrate (SMS) in corn silage represents a nutritionally balanced, environmentally friendly, and cost-effective feeding strategy for ruminants. This approach not only improves digestibility and energy availability but also contributes to methane mitigation and waste utilization, making it a viable alternative to traditional ruminant diets. This study investigated the effects of varying proportions of SMS and OS on total gas production, methane (CH₄) emissions, and dry matter degradability (DMD) of corn silage over different ensiling durations. A 6 × 4 factorial experimental design was employed, incorporating six dietary treatments with increasing SMS and OS levels (0%, 5%, 10%, 15%, 20%, and 25%) and four ensiling durations (0, 21, 42, and 63 days). Results indicated that increasing SMS and OS inclusion significantly reduced ($P < 0.001$) CH₄ emissions, with the highest reduction observed at 25% SMS and OS inclusion level. Total gas production ($P < 0.05$) and DMD ($P < 0.01$) were significantly influenced by both inclusion level and ensiling duration. Moderate inclusion levels (5% and 10%) resulted in higher DMD ($P < 0.01$), whereas levels above 20% negatively affected digestibility ($P < 0.05$). Longer ensiling periods (42 and 63 days) resulted in an increase in CH₄ production ($P < 0.001$) and DMD ($P < 0.01$). In conclusion, the inclusion of SMS and OS in corn silage-based ruminant diets decreases CH₄ emissions and increases digestibility at moderate (5% and 10%) inclusion levels. However, prolonged ensiling and higher SMS and OS proportions (greater than 20% inclusion) may compromise feed efficiency, necessitating careful consideration of inclusion levels and ensiling duration to optimize both environmental and nutritional benefits.

Impact of Ensiling on Nutritive Quality and Methane Emission from Molasses-treated *Gmelina arborea* Leaves for Sustainable Ruminant Production

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Keywords: Tropical legume forage, sweetener, rumen degradation, ruminants, ensiling technology.

Introduction

The impact of global warming as a result of the continued and uncontrolled release of gasses has implications on the small ruminant production and consequently food security. The responsibility of livestock production is to limit the carbon footprint thereby ensuring future sustainability. This study investigated effect of varying ensiling days on the chemical composition and methane production of *Gmelina arborea* leaves (GAL) treated with molasses feed for West African Dwarf goats

Materials and methods

The *Gmelina arborea* leaves were harvested from an already established field before fruiting, leaves were thoroughly washed to remove dirt and oven dried at 45°C for 1 hour to reduce the moisture content. Molasses was added to the prepared leaves at 10ml/kg and ensiled for 0, 3, 5, and 7 days in an anaerobic condition and incubated for 24 hours using in vitro gas production technique. Parameters assessed included; physico-chemical properties, anti-nutritional factors, CH₄, gas production, short chain fatty acids all carried out using standard procedures.

Results

Result showed that ensiling had significant effect on the chemical composition of molasses treated *Gmelina arborea* leaves (MTGAL). The highest crude protein was recorded in day 7 (9.27%) which was statistically different from the value recorded at 3 day which is at par with the unensiled GAL (UGAL) which had the lowest CP. As the fermented days progressed, the dry matter (DM) percentage and the Nitrogen Free Extract (NFE) values decreased. The percentage of the crude fibre was highest in *Gmelina arborea* leaves not ensiled (0 day) (3.98%) while ensiling days had effects on the crude fibre. Neutral detergent fibre was improved at 7 days MTGAL while cellulose was highest in the unensiled GAL. UGAL had the highest content of tannin, saponin, phytate and alkaloids. UGAL had the highest methane production after 24hours incubation. The day 7 ensiled MTGAL had the highest organic matter digestibility, SCFA, and IVDMD (16.97%, 0.06µm, 16.97%) respectively while MTGAL ensiled for 3 days had the least methane production throughout the incubation period

Conclusions

It could be concluded that fermentation of molasses treated *Gmelina arborea* for 7 days resulted in improved nutritional composition and reduced methane production thus has the potential of reducing enteric methane gas to the environment, thereby leading to an eco- friendly ruminant production when fed.

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Effect of Maturity on Yield, Nutritive Value and Fermentability of Whole-Crop White Lupin

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Keywords: dry matter loss, fermentability, nutritive value, lupin, yield.

Introduction

Home-grown high-protein forages have gained importance in ruminant nutrition, with silages produced from lucerne or clover, or in combination with grasses, being most important in temperate climatic regions. However, silage making from these forages involves cutting multiple times during the season, specific harvesting technology, and wilting in order to avoid effluent production and to improve the fermentation. Studies from UK and Italy (Fraser et al., 2005; Borreani et al., 2009) indicated that direct-cut whole-crop lupins had very low dry matter (DM) concentrations, and even wilting for up to 24 h did not increase it beyond 215 g kg⁻¹, thus resulting in high effluent production of up to 150 L t⁻¹ fresh matter. In addition, fermentation quality may also be poor, as reflected by very high butyric acid concentrations (Borreani et al., 2009). There has been an interest by farmers, in Germany, to use whole-crop annual legumes for silage production, but cut and ensiled directly. The aim of our study was to determine the optimal stage of maturity of white lupins in terms of yield, nutritive value and fermentability that ensures high silage quality without the risk of excessive effluent production.

Materials and methods

White lupin (*Lupinus albus*, cv. Celina) was sown on a farm in the German State of Saxony-Anhalt on March 25, 2024. The first harvest was done after 12 weeks of growth, on June 19, 2024, after flowering, and at fortnightly intervals thereafter. On each harvest, forage samples cut by hand at about 10 cm above ground were collected from the same four different field locations (initial plot size about 30 m²), were taken to the lab and chopped by a stationary chopper to 30 mm theoretical length of cut. The material from the two last harvests were processed a second time by a garden shredder to ensure that pods were properly crushed and kernels cracked. Forage was packed into 1.5 L-glass jars and stored for 111-132 days at room temperature (20-22 °C). Forage yields were determined by weighing the material harvested from 3-4 m². The nutritional composition of the forages was analyzed by official methods of the German Feed Evaluation System (VDLUFA; GfE, 2023), fermentability parameters by international standards, and losses during fermentation according to Weissbach (2005). Data were analyzed using the GLM procedure of SAS as a completely randomized block design. The REG procedure was used to describe the relationship between DM content at harvest and DM loss during fermentation. Differences among LSmeans were separated by Tukey test and significance declared at $P < 0.05$.

Results

Harvest date and stage of maturity affected all analyzed traits (Table 1). Most importantly, DM content showed a steep increase only after harvest 4, and higher crude protein and starch concentrations, organic matter digestibility (OMD), were observed, which explains the improvements in gross and metabolizable energy concentrations. The content of water-soluble carbohydrates markedly decreased after harvest 3. Based on fermentability coefficients, lupins from harvest 2, 3 and 5 were classified as easy-to-ensile, whereas those from harvests 1 and 4 as moderately difficult-to-ensile (Pauly and Wyss, 2019). In combination with low ash concentrations, silages from all forages may not have been at high or no risk of clostridia development and butyric acid formation. However, analysis of fermentation pattern is still in progress. Yields of DM, crude protein and energy were highest in forages from harvest 3 and 5, followed by that from harvest 4. In line with Fraser et al. (2005), The DM loss during fermentation declined with progressing maturity but were still >10% up to harvest 4. The relationship between DM at ensiling and DM loss during fermentation was best described by a power function ($y = 1,517 x^{-0.911}$, RMSE=1.99, $R^2_{adj}=0.846$, where y = DM loss, % and x = DM at harvest, g kg⁻¹ DM). From this model, a given increase in DM at harvest from 140 to 450 g kg⁻¹ results in an increase in DM loss during fermentation by 10% units.

Unfortunately, the lupin crop matured very rapidly after harvest 4 due to hot and dry weather. Therefore, the optimal stage of maturity (likely between harvest 4 and 5) could not be established. Forage from harvest 4 would still have produced high amounts of effluent (as it was observed during packing the jars), whereas material from harvest 5 was likely already too dry to enable proper pod and kernel processing and compaction under farm-scale conditions.

Table 1. Effect of forage maturity stage on yield, nutritive value, fermentability, and on dry matter loss of white-lupin silage (data given in g kg⁻¹ DM unless stated otherwise, n=4).

Item	Harvest					SEM	P	Contrast [‡]
	1	2	3	4	5			
Dry matter (g kg ⁻¹)	139c [†]	149c	152c	205b	597a	9.9	<0.001	L ^{***} , Q ^{***}
Crude ash	61a	48b	45c	46c	49b	0.4	<0.001	L ^{***} , Q ^{***}
Crude protein	162b	154b	154b	161b	198a	5.9	<0.01	L ^{***} , Q ^{***}
WSC ¹	112c	198a	158b	79d	51e	3.8	<0.001	L ^{***} , Q ^{***}
Starch	100e	103d	189c	192b	203a	0.4	<0.001	L ^{***} , Q ^{***}
Buffering capacity (BC) ²	45b	34c	30d	44b	55a	0.6	<0.001	L ^{***} , Q ^{***}
WSC/BC ratio	2.5c	5.8a	5.4b	1.8d	0.9e	0.09	<0.001	L ^{***} , Q ^{***}
FC ³	34c	61b	58b	35c	67a	1.0	<0.001	L ^{***} , Q [*]
OMD ⁴ (%)	64.0c	67.6b	67.0b	66.0b	70.5a	0.41	<0.001	L ^{***}
GE ⁵ (MJ kg ⁻¹ DM)	19.6b	19.2c	19.2c	19.8b	20.4a	0.05	<0.001	L ^{***} , Q ^{***}
ME ⁶ (MJ kg ⁻¹ DM)	9.8c	10.2b	10.1bc	10.3b	11.3a	0.09	<0.001	L ^{***} , Q ^{***}
Yield								
Dry matter (t ha ⁻¹)	6.91c	6.36c	12.42a	9.22b	12.11a	0.47	<0.001	L ^{***}
Crude protein (t ha ⁻¹)	1.11c	0.98c	1.91a	1.48b	2.38a	0.07	<0.001	L ^{***} , Q [*]
ME ⁶ (GJ ha ⁻¹)	67.53c	64.85c	125.25a	94.33b	137.26a	4.62	<0.001	L ^{***}
Dry matter loss (%)	16.0a	16.8a	NE	11.9a	4.7b	1.43	<0.001	

[†]Means within a row (harvest comparison) bearing unlike superscripts differ ($P < 0.05$).

[‡]Contrast for effect of harvest. L = linear, Q = quadratic, * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$.

¹water-soluble carbohydrates; ²g lactic acid kg⁻¹ DM; ³Fermentability Coefficient = DM (%) + 8 x WSC/BC ratio; ⁴Organic matter digestibility at feed intake level 1; ⁵Gross Energy; ⁶Metabolizable Energy, NE = Not Ensiled.

Conclusions

For a single-cut forage, white lupin may have potential as a silage crop due to high yield of DM, crude protein and metabolizable energy as well as good fermentability traits, given that it is harvested at a DM level that restricts effluent production and DM loss during fermentation. More studies are warranted focusing on ensiling the forage at DM levels ranging 30-50% using commercial harvest technology as well as feeding studies with silage from such material.

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Fermentation Quality of Lucerne as Affected by Year, Variety and Cut

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Keywords: fermentation quality, forage quality, dry matter loss, lucerne.

Introduction

Lucerne (*Medicago sativa*) silage plays an important role in ruminant nutrition in many regions of the world, mainly due to its high-protein concentration and fiber digestibility during optimal stage of maturity. However, adverse wilting conditions may result in too low a dry matter level at chopping, thereby increasing the risk of poor fermentation caused by clostridial activity (Auerbach et al., 2016). This risk is exacerbated by intentionally short wilting periods to reduce field losses. Our study aimed at testing the effects of year, variety, cut and their interactions on dry matter (DM) losses and fermentation quality of lucerne silage, which was ensiled without additives.

Materials and methods

Lucerne plots with two varieties (*Plato* and *Marshal*) were established on a dairy farm in the German State of Thuringia in autumn 2020 using a seeding rate of 30 kg ha⁻¹. Forages were cut about 10 cm above the ground at budding stage four times during 2021 and 2022, using with a sickle bar mower. After transport to the laboratory, forages were wilted outdoors on concrete for a maximum of 36 hours, chopped by a stationary chopper to about 30 mm theoretical length of cut, packed into 1.5 L glass jars and stored at 22 °C for 118-133 days. Nutrient composition and fermentability traits were analyzed by official methods of the German Feed Evaluation System. Fermentability coefficient (FC) was calculated as follows: DM (%) + 8 x water-soluble carbohydrate (WSC) content / buffering capacity (BC) ratio. In silages, DM losses were calculated and fermentation pattern was analyzed using routine chemical methods. Data were subjected to a three way-ANOVA factorial design ((2 (year) x 2 (variety) x 4 (cut)) using the GLM procedure of SAS. Means were compared by Tukey-test, and regressions were performed using the REG procedure to describe the relationships between silage variables. Significance was declared at $P < 0.05$.

Results

Many effects of year, variety and cut on chemical composition and fermentability traits of wilted forages were detected with numerous significant observed among factors (data not shown). The DM content after a short wilting phase was between 20.4 and 41.5%. The concentrations of crude ash ranging between 8.0 and 13.3% of DM, and those of crude protein varying between 19.5 and 25.2% of DM corresponded with typical values found in this forage in continental Europe. As expected, WSC concentrations were low (4.8-8.2% of DM) and BC was high, resulting in FC values of 24 to 48.

Data on DM loss and fermentation pattern of untreated lucerne silages are presented in Table 1. Low DM losses (<8%) were observed in silages from cuts 1, 2 and 3 in 2021 and from cut 3 in 2022, regardless of variety. With the exception of 3rd cut Marshal silages from 2021, NH₃-N levels were above 10% of TN, indicating a high degree of proteolysis even in silages with low DM losses. Silages from both varieties made from 2nd and 4th cut material in 2022 contained very low concentrations of lactic acid, which was accompanied by very high concentrations of butyric acid (>4.5% of DM). However, as shown for 4th cut material from 2021, high lactic acid concentrations can be found even if there was significant clostridial activity as reflected by butyric acid in excess of 1% of DM. In 11 out of the 16 treatments was ethanol found to be below 1% of DM, with the maximum content of 1.75% of DM detected in 4th cut silage from variety Plato in 2021.

Strong relationships were detected between various silage traits. Losses of DM during fermentation were closely related to butyric acid content ($y=5.91+2.97x-0.335x^2$, RMSE=1.27, $R^2_{adj}=0.836$, $P < 0.001$). Including more end-products of CO₂-releasing metabolic pathways, e.g. acetic and propionic acids and alcohols, did not improve the power of the relationship ($y=1.60+1.52x-0.054x^2$, RMSE=1.78, $R^2_{adj}=0.680$, $P < 0.001$). This may be explained by largely differing concentrations of other fermentation end-products at similar butyric acid levels, likely due to the activity of microorganisms other than clostridia. Butyric acid content, presented as the sum of n- and i-butyric acids, n- and i-valeric acids and n-caproic acid, was largely composed of n-butyric acid (data not shown in Table 1) as reflected by a strong linear correlation ($y=0.049-0.853x$, RMSE=0.275, $R^2_{adj}=0.979$, $P < 0.001$). Silage NH₃-N levels were linearly correlated with the concentration of butyric acid ($y=14.2+3.73x$, RMSE=4.59, $R^2_{adj}=0.759$, $P < 0.001$).

Table 1. Effects of year (Y), variety (V), cut (C) and their interactions on dry matter losses and fermentation quality of lucerne (LSmeans of 3 replicates per treatment, n=48)

Year	Variety	Cut	DM (%)	DML ¹ (%)	NH ₃ -N ²	pH	Lactate	Acetate	Butyrate ³	Ethanol
							----- % DM ⁴ -----			
2021	Plato	1	21.0h [†]	7.3e	20.6d	4.86g	8.66ab	5.72a	0f	0.69de
		2	24.9g	5.9f	15.3e	4.88fg	7.77c	3.74c	0f	0.62de
		3	41.3a	4.3g	10.1f	5.14de	4.62ef	1.24h	0f	0.48ef
		4	32.9def	9.4def	16.2e	5.36d	5.42e	1.83g	2.12d	1.75a
	Marshal	1	21.7h	7.7e	20.5e	4.80g	8.97a	5.24ab	0f	1.12c
		2	21.4h	6.4f	18.3de	5.09ef	8.11bc	4.82b	0f	0.67de
		3	43.1a	4.0g	8.7f	5.15de	3.89f	1.06h	0f	0.40f
		4	31.2f	8.7d	16.0e	5.12e	6.38d	2.50de	1.25e	1.71a
2022	Plato	1	34.2cde	13.7a	31.2b	6.20bc	1.55h	1.84fg	3.11c	0.80d
		2	33.8cdef	12.5b	29.8b	7.66a	0i	2.26efg	5.60a	0.69de
		3	36.0bc	6.0f	11.5f	4.76g	8.02bc	2.75d	0f	0.77d
		4	21.1h	12.3b	40.6a	6.26b	0i	4.84b	5.83a	1.38b
	Marshal	1	35.1cd	12.4b	26.1c	6.02c	2.79g	2.22efg	2.40d	0.74d
		2	31.8ef	10.7c	28.9bc	7.61a	0.42i	2.31def	4.60b	0.70de
		3	38.3b	5.7f	10.1f	4.77g	7.58c	2.34de	0f	0.68de
		4	21.5h	11.8b	32.4b	6.14bc	0i	4.77b	4.59b	1.19bc
SEM			0.50	0.15	0.67	0.043	0.155	0.092	0.120	0.042
Y			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.007
V			NS	<0.001	<0.001	0.017	0.002	0.008	<0.001	NS
C			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Y x V			0.038	<0.001	<0.001	NS	NS	0.004	<0.001	<0.001
Y x C			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
V x C			<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Y x V x C			NS	<0.001	<0.001	0.023	0.002	<0.001	NS	0.001

[†]LSmeans within a column bearing unlike superscripts differ ($P < 0.05$).

¹Dry matter loss during fermentation, ²% of total-N, ³Sum of n- and i-butyric acids, n- and i-valeric acids and n-caproic acid,

⁴corrected for the loss of volatiles during drying, NS = not significant.

Conclusions

Fermentation quality and in-silo DM losses can hardly be predicted due to numerous effects of year, cut and variety, and their interactions. Even when ensiled at reasonably high DM levels (30-40%), an unacceptably significant risk of clostridial activity persists, leading to poor fermentation quality and high DM losses. Especially when wilting is restricted to a short period (max. 36 hours) to preserve nutrients in the forage to be ensiled, additives need to be used strategically in order to inhibit undesired microorganisms, with the additive type – biological or chemical - being chosen based on prevailing harvest and wilting conditions.

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Fermentation Quality of Lucerne Silage as Affected by Variety and Additive Type

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Keywords: fermentation quality, inoculants, lucerne, nitrite, silages additives.

Introduction

Producing high-quality lucerne silage is key to its successful use in dairy rations but can be challenging due to generally low concentration of fermentable carbohydrates and high buffering capacity. Moreover, slow wilting rates under adverse weather conditions as well as limiting the wilting phase to maximal two days to minimize field losses, exacerbate the risk of poor fermentation quality and reduced nutritive value caused by clostridia development. Trials in our laboratories have shown a frequency of 50% clostridial silage when additives were not used. Thus, the aim of this study was to test the efficacy of different additive types in lucerne silage produced from two different varieties.

Materials and methods

Lucerne (*Medicago sativa* L.) leys were established on a dairy farm in the German State of Thuringia in summer 2020 using the varieties Plato and Marshal sown at 30 kg ha⁻¹. Material from the 1st cut in 2022 was harvested at budding stage by a sickle bar mower, wilted for 36 h in a barn and subsequently chopped by a stationary chopper to about 30 mm theoretical particle size. After contamination with soil (50 g kg⁻¹) to create challenging conditions, forages were treated with one of the following additives (n=3 per treatment): CON, untreated (tap water), LAB, lactic acid bacteria inoculant composed of *Lactobacillus buchneri* CNCM-I 4323 and *Pediococcus acidilactici* DSM 11673, added at a total inoculation rate of 1.67x10⁵ cfu g⁻¹, or NF, liquid blend of sodium nitrite (306 g L⁻¹) and sodium formate (206 g L⁻¹) at 2.5 mL kg⁻¹, subsequently packed into 1.5 L-glass jars and stored at 20-22 °C for 118 days. Forage (Table 1) and silage traits were analyzed using routine analytical methods. Statistical analysis was performed using the SAS (v4) procedures GLM, REG and NLIN. Differences among LSmeans were separated by Tukey test and significance declared at *P* < 0.05.

Table 1. Characteristics of wilted and chopped lucerne before soil contamination (n=3).

Variety	DM ¹	XA ²	XP ³	XF ⁴	WSC ⁵	BC ⁶	FC ⁷
Plato	318a [†]	105	229	235	80	79	40
Marshall	329b	102	234	232	82	82	41
SEM	0.1	0.4	0.3	0.5	0.3	1.1	0.3

[†]LSmeans within a column (cultivar comparison) bearing unlike superscripts differ (*P* < 0.05).

¹Dry matter, g kg⁻¹; ²Crude ash, g kg⁻¹ DM; ³Crude protein, g kg⁻¹ DM; ⁴Crude fiber, g kg⁻¹ DM; ⁵Water-soluble carbohydrates, g kg⁻¹ DM; ⁶Buffering capacity, g lactic acid kg⁻¹ DM; ⁷Fermentability Coefficient = DM + 8*WSC/BC.

Results

Additive use consistently and positively affected the fermentation process of slightly wilted as opposed to variety but many interactions among factors were observed (Table 2). Regardless of variety, untreated silage showed the highest in-silo losses, which was reduced by both additive, with the greatest effect observed for NF (Table 2). The degree of proteolysis was highest in untreated silage and could be reduced by additive use, especially by NF (across varieties, NH₃-N in % of TN: 33.0^a in CON vs 18.2^b in CE vs 11.5^c in NF, *P* < 0.001). The lowest concentration of lactic acid detected in untreated silage in silage from both varieties can likely be explained by anaerobic degradation of this acid by clostridia to form butyric acid, which exceeded 3% of DM. In combination with the highest ammonia-N levels, this caused pH values above 6.0, reflecting strong clostridial activity. Despite variety x additive interactions, positive effects of NF on these traits were more pronounced than those of LAB. Acetic acid production was lowest in NF silage from both varieties. Additive use largely restricted ethanol formation. When compared with other additive treatments across varieties, untreated silage contained the highest ethanol concentration (% of DM; 0.80^a in CON vs 0.51^b in CE vs 0.37^c in NF, *P* < 0.001). Despite significant differences between treatments, 1,2-propandiol was always detected at low concentration (<0.30% of DM).

Table 2. Effects of variety (V) and silage additive (A) and their interaction (V x A) on dry matter losses and fermentation quality of 1st cut lucerne silage (n=3, data given in % of DM unless stated otherwise).

Trait	Variety						<i>P</i> value			
	Plato			Marshal						
	Additive									
	CON ¹	LAB ²	NF ³	CON ¹	LAB ²	NF ³	SEM	V	A	V x A
Dry matter loss (%)	13.9a [†]	11.0b	7.3d	13.4a	9.7c	6.9d	0.14	***	***	*
NH ₃ -N, % of total-N	34.7a	20.2bc	11.8d	31.2a	16.1cd	11.2d	1.12	*	***	NS
pH	6.29a	5.50b	4.76d	6.57a	5.12c	4.70d	0.067	NS	***	***
Lactic acid	1.81d	3.32c	5.32ab	1.46d	4.52b	6.02a	0.184	**	***	**
Acetic acid	2.15ab	2.11b	1.39d	1.82c	2.28a	1.43d	0.032	NS	***	***
Propionic acid	0.87a	0.39b	0.02c	1.00a	0.08c	0c	0.048	NS	***	**
n-Butyric acid	2.69a	1.05b	0.08c	3.09a	0.27c	0.02c	0.160	NS	***	*
Butyric acid ⁴	3.12a	1.20b	0.08c	3.80a	0.28c	0.02c	0.170	NS	***	**
Ethanol	0.76a	0.51bc	0.32d	0.83a	0.51b	0.41cd	0.038	NS	***	NS
1,2-Propanediol	0.12c	0.20b	0.24ab	0.07c	0.29a	0.21b	0.014	NS	***	**

[†]LSmeans within a row not followed by the same letter differ ($P < 0.05$).

¹untreated, ²Hetero-/homofermentative lactic acid bacteria, ³Liquid blend of sodium nitrite and sodium formate, ⁴Σ of n-/i-butyric acids+n-/i-valeric acids+n-caproic acid, * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, NS = not significant.

Several strong correlations between numerous silage traits were detected based on the full data set (n=18). The DM losses were closely correlated with the total concentration of n-/i-butyric acids, n-/i-valeric acids and n-caproic acid ($y=7.42+4.01x-0.631x^2$, RMSE=0.803, $R^2_{adj}=0.917$, $P < 0.001$). By including other end-products from CO₂-generating microbial metabolic pathways, e.g. acetic and propionic acids and alcohols, the power of this relationship slightly improved to $R^2_{adj}=0.972$ ($y=1.66+2.630x-0.142x^2$, RMSE=0.471, $P < 0.001$). These findings confirm that CO₂ formation is the primary source for in-silo DM losses. Although it cannot be determined which type of clostridia – saccharolytic or proteolytic – were metabolically active and which type of substrate – sugar or lactic acid – was used, the strongly inverse relationship between lactic and butyric acid concentrations (Broken-Line-model, breakpoint for lactic acid concentration: 4.64 % DM, $R^2_{adj}=0.940$, RMSE=0.374, $P < 0.001$) led us to suggest that lactic acid was mainly utilized. There was a strong quadratic relationship between the total concentration of n-/i-butyric acids, n-/i-valeric acids and n-caproic acid and the ammonia-N level ($y=11.3+11.5x-1.6x^2$, RMSE=2.89, $R^2_{adj}=0.908$, $P < 0.001$) indicating strong activity of proteolytic clostridia especially in untreated silage from both varieties and, to a lesser extent, in LAB treated silage from variety Plato, which also contained i-butyric, n-/i-valeric and n-caproic acids, respectively.

Conclusions

Restricting the wilting phase of lucerne to a maximum of 36 hours to reduce field losses increases the risk of poor fermentation quality by clostridial activity due too to a low DM concentration at ensiling. As opposed to forage variety, additive use showed consistent effects on all tested traits, resulting in largely improved fermentation quality, and more efficient fermentation. Although both additive types can be recommended for lucerne, the chemical additive was superior to the LAB inoculant in terms of minimizing in-silo DM losses.

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Grass Silage for Biorefinery: Fermentation Quality and Nutritional Composition of Press Cake and Juice

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Keywords: feed preservation, liquid-solid separation, screw-press, formic acid, pulp, silage additive

Introduction

Grass represents a versatile, human-inedible biomass with multiple ecological benefits, making it a promising feedstock for green biorefinery applications. The green biorefinery process enables valorization of green biomasses, such as grass into diverse high value products. During this process, the solid (fibrous cake) and liquid fractions (protein-rich juice) can be utilized for feeding ruminants and monogastrics, among other applications (Rinne, 2024). This study aimed to evaluate the effects of fractionated or wilted grass treated with various additives on silage fermentation quality and their subsequent influence on liquid yield and composition of press juice.

Materials and methods

Pure stand of first regrowth timothy grass (*Phleum pratense*) cultivated in Jokioinen, Finland (60°48'N, 23°29'E) was harvested on 31 July, 2023, and precision chopped after mowing. Before ensiling, the fresh grass was divided into 3 biomass types: Fresh (grass as such), Press cake (residual solid fraction from grass pulping using a pilot single screw press [Smicon MAS SP300 filter press, Haderslev, Denmark]), and prewilted (Wilt; grass pre-dried for 5 hours using a forced-air open-circuit dryer at 30°C). An ensiling trial was conducted using cylindrical silos with three replicates per biomass type and three additive treatments: Control without additive (CON), lactic acid bacteria inoculant applied at 1 g/t (LAB; Kofasil Duo, Addcon, Bitterfeld-Wolfen, Germany [2×10^5 cfu/g fresh matter]) and formic acid-based additive applied at 5 l/t (FA; AIV 2 Plus Na, Eastman, Oulu, Finland). After 88 days of ensiling, silos were opened, weighed, and sampled for fermentation quality and aerobic stability analyses. Losses during fermentation were calculated based on weight loss and effluent losses. A 300 g batch of each treatment was pressed using a double screw press (Angel Juicer Ltd., Busan, South Korea) and chemical composition of the extracted juice was analyzed to calculate nutrient recovery. Experimental results were statistically analyzed using SAS PROC MIXED (SAS 9.4) with biomass type, additive and their interactions as fixed effects, and replicates as random effect. Statistical significance was considered at 5% probability, with pairwise comparisons performed using Tukey-Kramer test.

Results

Nutritional composition of the original biomasses before ensiling is presented in Table 1. Liquid yield obtained from pulping the grass biomass before ensiling was 0.24. Screw-pressing and drying increased the dry matter of Press cake and Wilt, while crude protein (CP) concentrations remained constant across all biomasses. Despite expectations of lower CP in Press cake due to liquid extraction, inefficiencies in the press likely prevented significant CP loss. Soluble compounds such as ash and water-soluble carbohydrates (WSC) were lower in Press cake due to partitioning into the liquid fraction.

Fermentation coefficient (FC) classified Fresh as difficult to ensile, whereas Press cake and Wilt fell within the intermediate ensilability category (Weissbach et al., 1974). Despite these differences, all silages exhibited good fermentation quality, with FA treatment further improving parameters such as reduced ammonia N levels, especially in Press cake (Table 2). The pH of Fresh and Press cake was below 4, while Wilt had slightly higher pH, likely due to lower water activity hindering the growth of lactic acid bacteria, as shown by reduced lactic and acetic acid production. Formic acid treatment restricted fermentation, resulting in lower lactic acid concentration compared to CON and LAB, and a release of WSC in Wilt silage (surpassing concentration before ensiling) likely due to acid hydrolysis of fiber (McDonald et al., 1991). Effluent loss was only observed in Fresh and highest loss was observed in FA treated silages. Simulating biorefinery performance of the silages using lab. scale double screw press resulted in lower liquid yield for Press cake and Wilt compared to Fresh. Pulping, wilting grass and FA treatment decreased the amount of CP retained in the press juice.

Conclusions

Good fermentation quality was achieved in all silages. Lactic acid bacteria inoculant boosted acetic acid fermentation, while formic acid restricted fermentation intensity and protein degradation. Pulping grass through biorefinery and subsequent ensiling offers a synergistic solution, optimizing silage production by mitigating fermentation losses while improving and stabilizing biorefinery products.

Table 1. Chemical composition of original biomasses prior to ensiling.

	Fresh	Press cake	Wilt
Dry matter (DM), g/kg fresh matter	195	230	301
Buffering capacity, g lactic acid/100 g DM	4.73	4.31	5.09
Fermentation coefficient	33	35	41
In DM, g/kg			
Ash	90	83	91
Crude protein	130	130	132
Water soluble carbohydrates	80	63	69
Nitrate-N	0.14	0.08	0.08
Neutral detergent fiber	596	597	578
<i>In vitro</i> organic matter digestibility, g/g organic matter	0.753	0.735	0.747

Table 2. Fermentation quality and biorefinery output of ensiled biomasses preserved without additive (CON) or with a lactic acid bacteria inoculant (LAB) or formic acid-based additive (FA).

Biomass (B)	Fresh			Press cake			Wilt			SEM	Significance ¹⁾		
Additive (A)	CON	LAB	FA	CON	LAB	FA	CON	LAB	FA		B	A	B×A
DM ²⁾ , g/kg	193 ^c	192 ^c	199 ^c	227 ^b	230 ^b	233 ^b	301 ^a	294 ^a	299 ^a	3.9	***	ns	ns
pH	3.93 ^{bcd}	3.95 ^{bcd}	3.95 ^{bcd}	3.74 ^d	3.99 ^{bc}	3.82 ^{cd}	4.43 ^a	4.11 ^b	4.58 ^a	0.050	***	ns	***
NH ₃ -N, g/kg N	62 ^a	65 ^a	34 ^c	52 ^{ab}	51 ^{ab}	20 ^d	51 ^{ab}	54 ^a	35 ^{bc}	2.7	**	***	*
Chemical composition, g/kg DM													
Ash	95 ^{abc}	99 ^a	94 ^{bc}	89 ^{de}	89 ^{de}	86 ^e	94 ^{bc}	96 ^{ab}	92 ^{cd}	0.8	***	***	ns
Crude protein (CP)	134	136	131	136	136	133	131	133	128	2.0	*	ns	ns
WSC ³⁾	25.4 ^b	15.6 ^b	39.1 ^b	6.0 ^c	0.2 ^f	0.4 ^e	1.6 ^d	0.6 ^e	135.7 ^a	5.76	***	***	***
Ethanol	7.6 ^{bc}	9.1 ^{abc}	11.2 ^a	8.9 ^{abc}	9.8 ^{ab}	7.8 ^{bc}	7.0 ^{cd}	6.9 ^{cd}	4.5 ^d	0.55	***	ns	***
Lactic acid	89.5 ^{ab}	70.7 ^{abc}	36.9 ^d	91.6 ^a	86.3 ^{ab}	49.1 ^{cd}	44.9 ^d	68.7 ^{bc}	8.8 ^e	4.46	***	***	**
Acetic acid	23.0 ^{cd}	40.7 ^a	17.3 ^d	22.8 ^d	32.2 ^b	20.2 ^d	16.9 ^d	29.0 ^{bc}	7.1 ^e	1.24	***	***	**
Propionic acid	0.08 ^b	0.05 ^b	0.05 ^b	0.29 ^a	0.74 ^a	0.06 ^b	0.03 ^b	0.03 ^b	0.08 ^b	0.131	***	ns	***
Butyric acid	0.10 ^b	0.08 ^b	1.08 ^a	0.08 ^b	0.04 ^b	0.11 ^b	0.07 ^b	0.03 ^b	0.05 ^b	0.231	***	***	**
Losses, g/kg DM	12.9 ^{bcd}	18.0 ^b	41.3 ^a	11.0 ^d	12.9 ^{bcd}	11.0 ^{cd}	12.5 ^{bcd}	16.7 ^{bc}	9.7 ^d	0.98	***	**	***
Aerobic stability, h	118 ^{ab}	141 ^{ab}	136 ^{ab}	166 ^{ab}	195 ^{ab}	236 ^a	134 ^{ab}	244 ^a	117 ^b	21.6	*	ns	*
Biorefinery output of ensiled biomasses													
Liquid yield, g/g	0.70 ^a	0.69 ^{ab}	0.71 ^a	0.63 ^c	0.63 ^c	0.63 ^{bc}	0.55 ^d	0.52 ^d	0.52 ^d	0.011	***	ns	ns
Liquid DM, g/kg DM	86 ^{bc}	77 ^d	84 ^{cd}	89 ^{bc}	85 ^{bc}	92 ^b	135 ^a	124 ^a	130 ^a	2.1	***	***	ns
Liquid ash, g/kg DM	173 ^c	183 ^{abc}	183 ^{abc}	189 ^{abc}	195 ^{ab}	179 ^{bc}	190 ^{abc}	201 ^a	197 ^{ab}	4.4	**	ns	ns
Liquid CP, g/kg DM	221 ^c	248 ^{ab}	193 ^{de}	237 ^b	256 ^a	182 ^e	220 ^c	243 ^{ab}	198 ^d	4.1	ns	***	***
Proportion of compounds captured in the liquid													
DM	0.31 ^a	0.28 ^b	0.30 ^{ab}	0.25 ^{cd}	0.23 ^{cde}	0.25 ^c	0.25 ^{cd}	0.22 ^e	0.22 ^{de}	0.005	***	***	*
Ash	0.57 ^a	0.51 ^{ab}	0.58 ^a	0.52 ^{ab}	0.51 ^{ab}	0.53 ^{ab}	0.50 ^{ab}	0.46 ^b	0.48 ^b	0.016	***	*	ns
CP	0.52 ^a	0.51 ^a	0.44 ^b	0.43 ^b	0.44 ^b	0.35 ^c	0.42 ^b	0.40 ^b	0.35 ^c	0.010	***	***	ns

¹⁾ ns = not significant (P>0.05); * P<0.05; ** P<0.01; *** P<0.001; ²⁾ Dry matter; ³⁾ Water soluble carbohydrates;

^{a-f} Means within the same row without same superscript differ significantly (< 0.05).

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Effects of Environmental Conditions and Management Practices on Epiphytic Microorganisms of Mixed Grasses and Alfalfa

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Keywords: Epiphytic microbes, Forage management, Lactic Acid Bacteria

Introduction

Epiphytic microflora comprise the naturally present communities of microbes on the surface of plants prior to harvest and ensiling. Plants have been found to excrete metabolites to promote epiphyte colonization and in return receive benefits such as protection against pathogens and various sources of abiotic stress, and assistance with nutrient acquisition (Li *et al.*, 2022). Epiphytic microorganisms are taxonomically diverse and include various lactic acid bacteria (LAB) (*Lentilactobacillus*, *Pediococcus*, *Lactiplantibacillus*, *Leuconostoc*, etc.) and taxa that are considered problematic during ensiling including *Enterobacteriaceae*, yeasts, molds, and spore-forming Clostridia and *Bacillus* (Lin *et al.*, 1992). Microbial composition on the plant surface can be highly variable and easily influenced by abiotic environmental factors such as temperature and precipitation. On-farm management practices, such as the application of organic fertilizer, crop species, and the frequency of cuts are also thought to affect microbial abundance and composition/diversity? Since epiphytic microflora exists on the plant surface, environmental factors contribute to a highly variable taxonomy even between plants of the same species throughout the growing season (Zhang *et al.*, 2022). This trial utilized quantitative polymerase chain reaction (qPCR) to examine targeted epiphytic microflora present on standing grass and alfalfa in response to management and extreme weather events throughout a single growing season.

Materials and methods

An 11-acre mixed field containing alfalfa and tall fescue designated for silage production was selected for this study located in Chazy, New York, USA (44.887663, -73.464803). Five 100m² plots were selected at random and recorded using a Garmin GPSMAP 65 (Garmin, Olathe, KS, USA). Sampling occurred when one or more of three defined criteria were met: one day prior to harvest, ten days post-organic fertilizer application, and following a specific weather condition based on six years of historical weather data. Briefly, a heat event was defined as three consecutive days with a maximum temperature $\geq 30^{\circ}\text{C}$, an extreme rain event was defined as ≥ 25.4 mm of precipitation in a 24-hour period, and three consecutive days with a maximum temperature $\leq 15^{\circ}\text{C}$ was defined as a cold weather event. Weather was monitored onsite and with a locally operated monitoring station. Samples from each of the 11 events were grouped by “occurrence” to chronologically denote their order throughout the season. During sampling, the center of each plot was located using GPS, and a 4m² plot was marked for sampling above 10 cm. Fresh forage was removed using a Craftsman 8” Hedge Trimmer (Stanley Black and Decker, New Britain, CT, USA) and placed into a clean bin while wearing gloves before being transferred for cutting and subsampling. After weighing and mixing, material was further cut to approximately 5 cm and subsampled for dry matter analysis and DNA extraction. Samples for DNA extraction were stored at -80°C , and dried samples were ground to 1mm and submitted for NIR analysis. Assessment of the epiphytic microflora was performed using real-time qPCR with primers to target total bacteria (16S), fungi, LAB, *Escherichia coli*, and *Salmonella*. Results were transformed to log copies per gram of fresh matter for analysis. Data were analyzed in R Studio (R Version 4.3.2). Normality was tested using a Shapiro-Wilk test and visualized with Q-Q plots. The non-parametric Friedman’s Test was used to analyze the qPCR targets across all samplings (occurrence) and blocked by plot. Selected NIR components including ash, acid detergent fiber (ADF), amylase-treated neutral detergent fiber corrected for organic matter (aNDFom), Crude Protein (CP), fat (ether extract) and water-soluble carbohydrates (WSC) were similarly analyzed across all samplings and blocked by plot. Finally, Spearman correlation analysis was performed on the qPCR targets, NIR results, weather conditions, and management practices. Strong correlations ($\geq 0.7/ -0.7$) were statistically analyzed with a t-test using the `corr.test` function in R.

Results

Throughout the 2024 growing season (May to October) there were 5 total harvests. There was 1 recorded heat event where a precipitation event immediately followed. There were 8 total rain events during the growing season, and samples were collected following 3 of those events.

The qPCR analysis on the forage samples collected at each of the occurrences indicate that *Salmonella* remained undetected, and copies belonging to *Escherichia coli* fell below the limit of detection. Total bacteria (16S) copies were consistent throughout the growing season ($P \geq 0.05$). Total fungi did experience significant differences between occurrences 1, 9, and 10 ($P = 0.03$). Average fungal copies by plot were highest at two harvest events (4.02 and 4.28 for occurrences 1 and 9 respectively). Furthermore, total copies of lactic acid bacteria (LAB) experienced significant differences between occurrences 6 and 11 ($P = 0.01$). Copies belonging to LAB were highest during a mid-season harvest (occurrence 6, 3.88 copies), with copies falling below the limit of detection at the final harvest of the season (occurrence 11, 2.46 copies).

Total fiber (ADF and aNDFom) was increased at first harvest (occurrence 1; $P < 0.001$ and $P = 0.02$ respectively), whereas ash and CP were lowest at this occurrence ($P = 0.003$ and $P < 0.001$ respectively). Ash concentrations, however, were highest following organic fertilizer application (occurrence 2). From the sampling that followed a

combined fertilization and precipitation event (occurrence 5), CP was highest whereas ether extract and WSC were lowest ($P < 0.001$ respectively). In contrast, the following harvest (occurrence 6) had greater concentrations of WSC.

Copies belonging to LAB were positively correlated with ADF from plot 1 ($r^2 = 0.72$) across all occurrences ($P = 0.012$). Copies of fungi were negatively correlated with the dry matter content in plot 5 across all occurrences ($r^2 = -0.80$; $P = 0.003$). Additionally, copies belonging to LAB were positively correlated with maximum air temperature across harvests ($r^2 = 0.74$, $P < 0.001$).

Conclusions

Total bacteria (16S), fungi, and LAB comprised the epiphytic microbes that were consistently detected in all samples throughout the 2024 growing season. Copies of LAB seem to be most affected by maximum air temperature, however, total fiber content in forages seems to be a contributing factor as well. In this growing season, copies belonging to total fungi were highest during two harvests in which the maximum air temperatures ranged from 19°C to 23°C. In contrast, total fungi copies were lowest following application of organic fertilizer. Furthermore, it seems fungi is most affected by higher dry matter concentrations. Differential responses were observed in microbial populations that contribute to silage quality such as fungi and LAB in response to management practices, weather conditions, and nutritional composition. Therefore, further research is needed to better characterize the relationships that affect the epiphytic microflora throughout growing seasons, including community analysis using metagenomic approaches.

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Extraction Technique Influences Recovery of Volatile Fatty Acids in Corn and Grass Alfalfa Mixed Silage

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Keywords: Silage VFAs, Incubation time, VFA extraction

Introduction

Volatile fatty acids (VFA) are short chain fatty acids produced during anerobic fermentation by microorganisms, and they serve as key indicators of the quality of ensiling. As a strong acid, lactate contributes to ensiling by quickly decreasing the pH (Rooke and Hatfield, 2003). While acetate and propionate are not as strong as lactate, they have been postulated to improve silage stability during aerobic feed-out by providing antifungal properties (Muck *et al.*, 2018). In contrast, butyrate in silage generally indicates poor fermentation from undesirable microorganisms. Increased quantities of butyrate can contribute to higher dry matter losses, instability, and potentially present a pathogenic threat when offered to animals (Pahlow *et al.*, 2003). Hence, proper analysis of VFAs is important for providing accurate results that inform producers on the quality of their silage.

Furthermore, the method of extraction can affect the concentration of VFAs and subsequent interpretation. The first step of silage VFA extraction involves incubation/ soaking into solution. The time of soaking is not well defined with some sources citing 2-3 hours, while others recommend an overnight soak at 4°C. Since incubation at 4°C will not inhibit microbial activity, and the prolonged soak times allow for evaporation of some VFAs, it is plausible to expect differences as a result from soak times. Therefore, we have implemented an extraction technique that reduces the soak time to mitigate these potential limitations of existing techniques. In this experiment, we sampled corn silage and grass alfalfa mixed silage from experimental bunkers for the purpose of comparing the two different methods of VFA extraction, benchmarked against commercial laboratories in North America.

Materials and methods

Corn silage and grass silage were sampled at two heights (50 and 150 cm below the surface) on separate occasions from experimental bunkers at the William H. Miner Agricultural Institute (Chazy, NY, USA). Following removal from each height, both piles were then divided into five equal piles on a clean tarp, individually and thoroughly mixed before collecting subsamples (10 samples per bunker). All samples were analyzed for the following: dry matter analysis at 55°C for 48 hours, two methods of in-house VFA extraction, and external analysis at four different forage laboratories. All subsamples for in-house extraction were frozen at -20°C, and subsamples for commercial analysis using wet chemistry of fermentation profiles (Labs 1, 2, 3, and 4) were shipped overnight on ice.

For in-house VFA extraction, the two methods differ in the amount of silage and water used for extraction, as well as the duration and conditions for soaking, and filtration. The first method (M1) uses a longer soaking time with a minimum 1 hour at 4°C and 1 hour in a 25°C water bath, as based off Fussell and McCalley (1987) with modifications. M1 utilizes 100 g of silage in 200 mL of milliQ water. Following the two 1-hour incubations, samples were then filtered through four layers of cheesecloth and centrifuged at 447 xg for 15 minutes. Supernatants were filtered through a 25mm Whatman filter and measured for pH before storage at -20°C. For the second method (M2), 40 mL of milliQ water was added to 11 g of silage in 50 mL conical tubes before being placed in a 28°C water bath and sonicated for 1 hour. Samples were similarly filtered, measured for pH, and stored at -20°C.

All extracts were analyzed using HPLC (lactate, acetate, 1,2-propanediol, and ethanol), GC-MS (propionate, butyrate), and for ammonia using a microplate assay. Wet chemistry results from the four commercial laboratories and the two extraction techniques were converted to g per kg on a dry matter basis for analysis.

Finally, the growth of yeasts and molds were evaluated with varying incubation times from M1. Briefly, samples of corn and grass silage were similarly collected. Fresh samples (0 hours) were enumerated for yeasts and molds on dichloran Rose Bengal chloramphenicol culture media after collection. The remaining samples were incubated at 4°C for either 1, 5, 11, or 23 hours, plus one additional hour at 25°C. Samples were then enumerated after a total of 2, 6, 12, and 24 hours of incubation. All plates were incubated 28°C for 48 hours.

Data analysis was performed in R Studio (R Version 4.3.2). Comparison of the extraction techniques were evaluated by forage type and bunker sampling height through a Kruskal-Wallis test for the VFAs, ethanol, and ammonia. Results from the four commercial laboratories were also analyzed by forage type and bunker sampling height with a Kruskal-Wallis test, followed by a Dunn's post-hoc test. Results from the extraction technique and four commercial labs were compared through Principal Components Analysis (PCA) by forage type and bunker sampling height. Yeast counts across the different forage types and soak timepoints were analyzed with repeated measures ANOVA in R.

Results

Yeast counts increased with longer soak durations for both corn and grass silage samples ($P = 0.009$). The largest numeric increases were observed after 2 hours and 24 hours for corn and after 2, 12, and 24 hours for grass.

M2 increased the recovery of lactate, acetate, ammonia, and ethanol in corn silage samples at both sampling heights ($P \leq 0.05$). The recovery of 1,2-propanediol was only increased in M2 corn samples collected at the 150 cm depth ($P \leq 0.05$). In grass, M2 only increased the recovery of ammonia in samples collected from the 50 cm depth ($P = 0.009$), however, samples from the 150 cm depth had a greater recovery of lactate, acetate, 1,2-propanediol, ethanol, and ammonia ($P \leq 0.05$).

Although VFA concentrations within forage types were numerically similar across labs, there were some significant differences within forage types and sampling depths. For instance, Lab 3 reported higher concentrations of lactate, acetate, and propionate in corn silage sampled from the 50 cm ($P \leq 0.05$). Lab 3 also reported higher concentrations of lactate in corn silage samples collected at 150 cm ($P = 0.01$). In grass silage samples, Lab 2 reported higher concentrations of lactate, acetate, and propionic acid from the 50 cm depth ($P \leq 0.05$). However, Lab 1 reported higher concentrations of lactate, acetate, and 1,2-propanediol in grass silage collected from the 150 cm depth ($P \leq 0.05$). Additionally, Lab 4 reported lower concentrations of lactate in grass collected at the 150 cm depth compared to the other three labs ($P = 0.001$).

When comparing the two extraction techniques to the four commercial labs, PCA of the corn samples collected from 150 cm indicate M1 concentrations are most dissimilar compared to M2 and the four commercial labs. Additionally, PCA of the grass samples collected from 150 cm are more distinct by lab and extraction technique.

Conclusions

The recovery of VFAs across samples using M1 were lower compared to M2. It appears the duration of incubation has a negative impact on the recovery of VFAs, including those that are more prone to volatilization. Furthermore, sonication seems to be an appropriate alternative to prolonged incubations. Despite differences across the four commercial labs, some were numerically minimal. Furthermore, samples from M1 were more distinct when compared to M2 and some of the commercial labs. These results imply the technique of M2 is more aligned with commercial analysis. M2 not only improved the recovery of VFAs but is also more efficient with respect to time and amount of material required. Finally, these findings further iterate the importance of extraction technique for proper interpretation of fermentation results.

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Inoculant Effects on Bacterial Community, VOC Production, Aerobic Stability, and Fiber Degradability of Alfalfa-Grass Silage

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Keywords: fiber degradability, *Lentilactobacillus buchneri*, *Lentilactobacillus hilgardii*

Introduction

Mixed silage containing alfalfa and grass can face challenges with aerobic stability when ensiled at a high DM content. Inoculants combining facultative and obligate heterofermentative lactic acid bacteria (LAB) offer a dual benefit by improving fermentation and enhancing aerobic stability (Muck et al., 2018). The inclusion of exogenous fibrolytic enzymes to bacterial inoculants can aid in the release of sugars that fuel fermentation, while improving fiber accessibility to microorganisms (Zhu et al., 2011). In this context, volatile organic compounds (VOCs) serve as valuable indicators of fermentation quality and spoilage processes, as their production is closely linked to microbial activity. This study evaluated the effects of an inoculant on the fermentation, bacterial community, aerobic stability, NDF degradability (NDFD), and VOC profile of alfalfa-grass silage, compared to untreated and chemically treated silages.

Materials and methods

A mixture of alfalfa and grass was harvested with a DM content of 35.9% and treated with: water (CTRL); an inoculant containing *Lentilactobacillus buchneri* NCIMB 40788 at 75,000 cfu/g of fresh forage, *Lentilactobacillus hilgardii* CNCM I-4785 at 75,000 cfu/g of fresh forage, *Pediococcus pentosaceus* NCIMB 12455, at 100,000 cfu/g of fresh forage, xylanase, and beta-glucanase (INO, Lallemand Specialties Inc., Milwaukee, USA); a chemical additive containing sodium benzoate, sodium propionate, and sodium nitrite at 3 L/t of fresh forage (CHEM). Forage was packed into 7-L buckets (5 replicates per treatment) at approximately 190 kg/m³ of DM. Silos were stored at 22 ± 2°C for 0, 2, 5, 10, 20, 40, 80, or 160 d. Data were analyzed for pH, concentrations of lactic acid, acetic acid, 1,2-propanediol, and ethanol using HPLC, aerobic stability, bacterial community composition by 16S rRNA analysis, and headspace VOC analyzed by GC/MS. *In situ* NDF degradability (NDFD) in 24, 30, 48, and 240 h was only analyzed for CTRL and INO at 160 d. Aerobic stability was defined as the number of hours it took for the silage mass temperature to rise 2°C above the ambient temperature. Data were analyzed separately for each ensiling duration using Kruskal-Wallis test, followed by Dunn's post hoc test with *P*-values adjusted (*Padj*) using the Benjamini-Yekutieli method in R (version 4.2.3). Differential abundance analysis of the bacterial community composition and VOC data was performed using DESeq2 (version 1.38.2) in R. Significance was declared at *Padj* < 0.05.

Results

Inoculated silage exhibited a higher abundance (log₂ fold change) of *Lentilactobacillus* compared to both CTRL and CHEM at 2 d (10.65 and 10.64, respectively), 5 d (9.22 and 13.52), 10 d (6.55 and 5.70), 20 d (6.54 and 4.66), 40 d (5.14 and 4.20), and 80 d (1.44 and 2.00). Similarly, INO had a higher abundance (log₂ fold change) of *Pediococcus* compared to CTRL at 2 d (6.13), 5 d (4.68), 10 d (3.15), 20 d (2.56), 40 d (2.30), and 80 d (3.46). The abundance of *Pediococcus* was also greater in INO compared to CHEM at 2 d (5.64), 5 d (3.58), 10 d (3.23), 20 d (2.29), 40 d (1.90), 80 d (2.24), and 160 d (6.36). These findings highlight the successful proliferation of INO, as both *Lentilactobacillus* and *Pediococcus* are genera of the strains present in the inoculant. Inoculants with facultative heterofermentative LAB like *P. pentosaceus* are used to promote a more rapid and pronounced pH reduction (Muck et al., 2018). INO had a lower pH compared to the CTRL at 2 d (5.54 vs. 5.94), 10 d (4.27 vs. 4.84), 20 d (4.28 vs. 4.69), 40 d (4.32 vs. 4.61), 80 d (4.28 vs. 4.56), and 160 d (4.28 vs. 4.49). Similarly, INO achieved a lower pH than CHEM at 2 d (5.54 vs. 5.91), 5 d (4.33 vs. 5.26), and 10 d (4.27 vs. 4.83). Although INO had similar lactic acid concentrations to CTRL at all ensiling durations, it showed higher lactic acid concentrations (g/kg of DM) compared to CHEM at 2 d (22.49 vs. 1.31), 5 d (70.14 vs. 23.11), and 10 d (76.87 vs. 41.52).

1,2-propanediol is a known marker of *L. buchneri* and *L. hilgardii* activity (Muck et al., 2018). Its levels (g/kg of DM) were higher in INO than CTRL from 20 d onward (20 d: 10.07 vs. 0.89, 40 d: 11.65 vs. 3.57, 80 d: 3.51 vs. < 0.01; 160 d: 6.15 vs. 0.57) and higher than CHEM at 0 d (0.69 vs. 0.14) and 80 d (3.51 vs. < 0.01). During the early stages of ensiling, acetic acid is mainly produced by enterobacteria (Kung et al., 2018). HPLC analysis showed that INO silage had lower acetic acid levels (g/kg of DM) than CTRL at 5 d (5.25 vs. 12.25) and 10 d (7.15 vs. 12.29) but higher levels than CHEM at 2 d (5.21 vs. 1.94). Additionally, INO had lower abundances (log₂ fold change) of *Enterobacteriaceae* compared to CTRL at 5 d (-2.17), 10 d (-2.13), 20 d (-2.23), 40 d (-2.16) and 80 d (-2.71). However, INO had higher *Enterobacteriaceae* abundances when compared to CHEM at 2 d (1.89), 5 d (2.25), 10 d (2.88), 20 d (2.40), 40 d (3.01), and 160 d (3.24). These results suggest that while INO suppressed the activity of enterobacteria, it was less effective than CHEM. In the latter stages of ensiling, the production of acetic acid is typically attributed to obligate heterofermentative LAB activity (Kung et al., 2018). Headspace VOC analysis revealed that INO silages had higher acetic acid levels (log₂ fold change) than CTRL at 20 d (1.22), 40 d (1.04), 80 d (1.32), and 160 d (1.92). INO also had higher acetic acids levels than CHEM at 80 d (1.19) and 160 d (1.81).

Ethanol concentrations were similar between INO and CHEM. However, INO had lower ethanol levels (g/kg of DM) than CTRL at 5 d (2.58 vs. 5.64), 10 d (3.31 vs. 7.32), 20 d (2.44 vs. 7.67), and 160 d (2.68 vs. 5.55). Ethanol is mainly produced by yeasts and enterobacteria (Kung et al., 2018), therefore the decrease in ethanol levels suggests that inoculation suppressed the growth of these microorganisms. This assumption is supported by the fact that yeasts were undetectable in INO and CHEM ($< 1.00 \log \text{ cfu/g}$ of FM) but present in CTRL ($3.66 \log \text{ cfu/g}$ of FM) at 20 d. Also, the earlier-discussed inhibition of enterobacteria aligns with this finding. Once the silage is exposed to air, yeasts can metabolize lactic acid, initiating aerobic deterioration (Kung et al., 2018). At 2 d, CHEM exhibited greater aerobic stability (h) than CTRL (10 vs. 0). By 5 d, INO demonstrated significantly higher aerobic stability (h) than CTRL (204 vs. 5) and showed numerically higher stability than CHEM (204 vs. 12). At 10 and 20 d, both INO and CHEM ($> 240 \text{ h}$) had higher aerobic stability than CTRL (203 h at 10 d and 151 h at 20 d). By 40 d of ensiling, all treatments displayed similar aerobic stability. At 160 d, INO silage had a higher NDFD (% of NDF) in both 48 h (73.38 vs. 67.97% of NDF) and 240 h (80.42 vs. 75.85% of NDF) compared to CTRL.

Acetone levels ($\log_2$ fold change) were lower in INO than in CTRL at 20 d (-11.88) and 40 d (-12.80), and also lower than in CHEM at 20 d (-10.11). Similarly, INO had lower levels ($\log_2$ fold change) of 2-butanone than CTRL at 10 d (-13.14), 20 d (-9.84), 40 d (-17.59), 80 d (-18.98), and 160 d (-18.97). Furthermore, INO showed higher levels ($\log_2$ fold change) of 2-butanone at 2 d (11.47), 5 d (12.07), and 20 d (7.51) compared to CHEM, but lower levels at 80 d (-16.99) and 160 d (-16.26). Acetone and 2-butanone are associated with undesirable fermentation, such as that caused by enterobacteria. Alcohols, mainly produced by yeasts and enterobacteria, can react with acids to form esters (Kung et al., 2018). INO silages had lower levels of propyl acetate ($\log_2$ fold change) than CTRL and CHEM at 80 d (-2.78) and 160 d (-1.71). Additionally, INO had lower levels of ethyl hexanoate ($\log_2$ fold change) than CTRL at 10 d (-1.79), 20 d (-3.04), 40 d (-1.88), and 160 d (-1.71), and compared to CHEM at 5 d (-1.28), 10 d (-2.28), 20 d (-2.97), and 40 d (-1.57). Regarding ethyl acetate, INO had lower levels ($\log_2$ fold change) than CTRL at 10 d (-1.82) and 20 d (-1.11), but higher levels than CHEM at 2 d (1.99) and 5 d (1.02). Lastly, INO had higher methyl acetate levels ($\log_2$ fold change) than CTRL at 160 d (1.56) and higher levels than CHEM at 5 d (1.05) and 160 d (1.04).

Conclusions

The inoculant effectively dominated the bacterial community, accelerating the pH reduction. It also suppressed *Enterobacteriaceae* and inhibited yeasts while enhancing aerobic stability. The inoculant reduced the production of VOCs associated with undesirable fermentations like 2-butanone, acetone, and esters. The improvements in NDFD with inoculation suggest a positive effect on the fiber degradability.

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Is Wilted Grass Silage a Good Option for Commercial Feedlots?

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Keywords: feedlot, haylage, wilting

Introduction

Integrated crop-livestock systems have been adopted by Brazilian farms in recent decades. In such systems tropical grasses have been used for both cover crops and silage making. Hence, grass silage has become an important fiber source for feedlots. Grasses are typically harvested at low DM (20-25%), which affects DM yield, silage fermentation, and intake (Wright et al., 2000). An alternative to overcome this issue has been wilted silage (WS), since it improves silage quality by increasing DM. Although wilting grass prior to ensiling has generally increased DM intake (DMI), poorer feed conversion efficiency and no increase or depression in performance have been reported (Steen, 1984; Wright et al., 2000). These response patterns are very relevant to feedlots operations, since a large number of animals are slaughtered annually. Thus, the objective of this study was to evaluate the influence of direct cut grass silage (DC) and WS on intake, performance, and carcass traits in finishing bulls when fed at 15% DM inclusion in diets.

Materials and methods

The experiment was conducted at a commercial feedlot in the county of Araguaiana, MT, Brazil (15°38'43"S, 51°52'54"W, and altitude of 319 m). Treatments consisted of two finishing diets with the following sources of fiber: DC silage or WS included at 15% of the diet (DM basis). One hundred and sixty hectares of Miyagi grass (*Panicum maximum* cv. Miyagi) was planted in a center pivot in February 2024. In late April 2024, half of the field was harvested as DC silage (24% ± 1.4% DM), and half was harvested as WS (45% ± 3.1% DM). The DC harvest was made using a self-propelled forage harvester (Claas Jaguar 850), set at 15 mm theoretical length of cut (TLC), and equipped with direct disc header. For WS, the forage was mowed (Krone EasyCut TC 320 CV), tedded (Krone Vendro 790), and raked (Krone Swadro TC 640). After raking, a self-propelled forage harvester (Krone BIG X 630), set at 8 mm TLC, and fitted with a pickup header (Krone EasyFlow 300) was used to harvest the wilted forage. Both silages were inoculated with an inoculant containing *Enterococcus faecium*, *Lactobacillus plantarum*, and *Lactococcus lactis* at a commercial dose. The DC and WS silages were stored for 62 days in separate side-by-side pile silos until the feeding trial was initiated. A total of 2,250 Nellore bulls (initial shrunk BW = 400 ± 27.0 kg) were randomly assigned to one of two treatments (112 bulls per pen; 10 replicates per treatment). The diets (DM basis) consisted of grass silage (DC or WS; 15%), high-moisture corn (35%), ground dried sorghum (13.7%), DDGS (33%), urea (1.2%), and mineral/vitamin supplement (2.1%). Treatment diets were fed for 105 d. The diets were formulated using the NASEM (2016) to provide an average daily gain (ADG) of 1.4 kg/d. Throughout the experimental period, the diets were delivered twice a day (at 0800 and 1500 h) in equal proportions, as a total feed mixture. The DMI was calculated as the total kg of DM delivered to each pen after subtracting the weight of dry orts. The DMI fluctuation was calculated as the difference in intake between consecutive days (Bevans et al., 2005). On the day of shipping to slaughter, the final BW (FBW) was recorded, and bulls were shipped and harvested the next day at a commercial beef processor. Hot carcass weight (HCW) was assessed immediately following the harvest procedure, and dressing was calculated as HCW/SBW, in which SBW is the shrunk body weight. Data was analyzed as a completely randomized design using the MIXED procedure of SAS. Pen was considered the experimental unit. The statistical model considered the fixed effects of the treatment (DC vs. WS diets) and pen nested within the treatment as a random effect. An α of 0.05 determined significance and an α of 0.06 to 0.10 was considered a tendency.

Results

The DM concentrations of DC and WS silages were 23.1 ± 2.1% and 47.7 ± 2.8%, respectively. The pH values were 5.6 ± 0.2 and 4.9 ± 0.2 for DC and WS, respectively. For DC silage, the concentrations of lactic acid, acetic acid, butyric acid, and propionic acid were 1.74 ± 0.2%, 2.79 ± 0.5%, 2.12 ± 0.2%, and 0.41 ± 0.1%, respectively. Butyric acid was not found in WS and the concentrations of other acids (lactic, acetic, and propionic) were 3.07 ± 0.4%, 1.72 ± 0.2%, and 0.13 ± 0.1%, respectively. As expected, the WS was well preserved, as evidenced from the absence of butyric acid and lower pH values.

Cattle fed WS diet had a greater DMI ($P = 0.03$) and lower gain:feed ratio ($P = 0.01$) compared with bulls fed with DC diet (Table 1). The 6.6% increase in DMI due to wilting can be considered a common result. Other studies which evaluated the effects of WS in beef cattle diets also reported greater DMI for WS compared to diets with unwilted silage (Wright et al., 2000). The poorer fermentation in DC silages may be linked to a lower DMI in this treatment. The DMI fluctuation was not impacted by the diets ($P = 0.21$) but bulls fed DC diet tended ($P = 0.08$) to have greater orts. Although treatments have affected intake, no differences were obtained for FBW, ADG, HCW, and dressing. No effect on performance sustained by WS diet is in agreement with other results which have generally shown that WS sustains similar or lower gain than DC diet in spite of a greater DMI of the wilted forage (Steen, 1984). It has been suggested that this response could reflect lower nutrient intakes, which can often arise from the loss of available nutrients during wilting, and which are not totally compensated for by the increased DMI (Wright et al., 2000).

Table 1. Intake and performance of Nellore bulls fed finishing diets containing direct-cut grass silage (DC) or wilted grass silage (WS) at 15% DM inclusion.

Item	Treatment		SEM	P-value
	DC diet	WS diet		
DM intake, kg/d	9.1	9.7	0.18	0.03
DM intake, % BW	2.0	2.1	0.04	0.03
DM intake fluctuation, kg	0.31	0.25	0.26	0.21
Orts, kg/animal/d	1.28	1.04	0.09	0.08
G:F	0.15	0.14	0.002	0.01
FBW, kg	532	533	2.61	0.82
ADG, kg/d	1.34	1.34	0.02	0.81
HCW, kg	319	318	1.37	0.78
Dressing, %	60	60	0.11	0.22

IBW = initial body weight; FBW = final body weight; ADG = average daily gain; G:F = gain:feed ratio; HCW = hot carcass weight

Conclusions

It is concluded that WS increased DMI but had no effects on performance when fed to finishing bulls at 15% dietary DM inclusion. These results led to a lower gain:feed ratio for WS diet. For feedlots considering adopting WS, other factors such as silage losses, costs, and logistics should be evaluated for the technology to be viable.

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Effect of Inoculant on Alfalfa Silage Challenged with Air Stress and Loosely Packed Conditions

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Keywords: Lactic acid bacteria (LAB), fermentation, bacterial communities, and fungal communities

Introduction

Compared to grain silages, alfalfa has a low level of available water-soluble carbohydrates and high buffering capacity, which makes it more difficult to ensile (Borreani et. al., 2018). Use of combination heterolactic and homolactic lactic acid bacteria (LAB) silage inoculants helps to speed fermentation and reduce growth of undesirable bacteria, yeasts, and molds, enhancing overall silage quality and aerobic stability (Muck et al., 2018). However, laboratory ensiled alfalfa typically will not spoil, making it difficult to assess the effect of combination LAB inoculants on aerobic stability. The objectives of this study were:

- 1) To assess the ability of two independent ensiling challenges, low packing density and repeated aerobic exposure, to result in aerobic spoilage in a laboratory setting,
- 2) To assess the ability of a mixed homolactic and heterolactic inoculant to overcome those challenges, and
- 3) To characterize the effects of the challenges and inoculant on bacterial and fungal communities.

Materials and methods

Alfalfa forage was harvested, wilted to 39% dry matter (DM), chopped, and packed into 7.5 L bucket silos. Treatments were applied according to a 2×2×2 factorial design (with 5 replicates per treatment) as follows:

1. Inoculant type (**INO**): treatment with water (**CON**) or 300,000 CFU/g of a mixture of *Lentilactobacillus buchneri*, *Lactiplantibacillus plantarum* and *Lactococcus lactis* (**BA**, Bonsilage Alfa, Provita Supplements),
2. Air stress (**AIR**): ensiled buckets were subjected to no air stress (**NAS**) or air stress (**AS**) where the bucket silo had 1.6 cm diameter holes (2 at the bottom and 1 at the top plugged with rubber stoppers) that were opened (stoppers removed) for 24 h every 14 d beginning after 2 weeks of ensiling, and
3. Packing density (**D**): bucket silos were packed to low density of 181 kg/m³ (**LD**) or normal density of 239 kg/m³ (**HD**).

Silos were stored at 30°C and opened after 112 d. Organic acids, DM recovery, pH, and LAB and mold counts were measured following opening. Silage was then exposed to air and the number of hours before the temperature rose 2°C above the baseline was considered as aerobic stability. Data were analyzed by JMP Pro17, using the Fit Model considering the main factors (**INO**, **AIR**, **D**) and their interactions. Microbial pellets collected from silage were used for DNA extraction with the QIAGEN PowerFecal Pro DNA kit, and 16S/ITS sequencing was performed by RTL Genomics (Lubbock, TX, USA) using Illumina MiSeq. Sequenced FASTQ files were imported into QIIME 2 to clean and filter raw sequences to identify taxonomical details, while R Studio was used for the visualization and statistical analysis.

Results

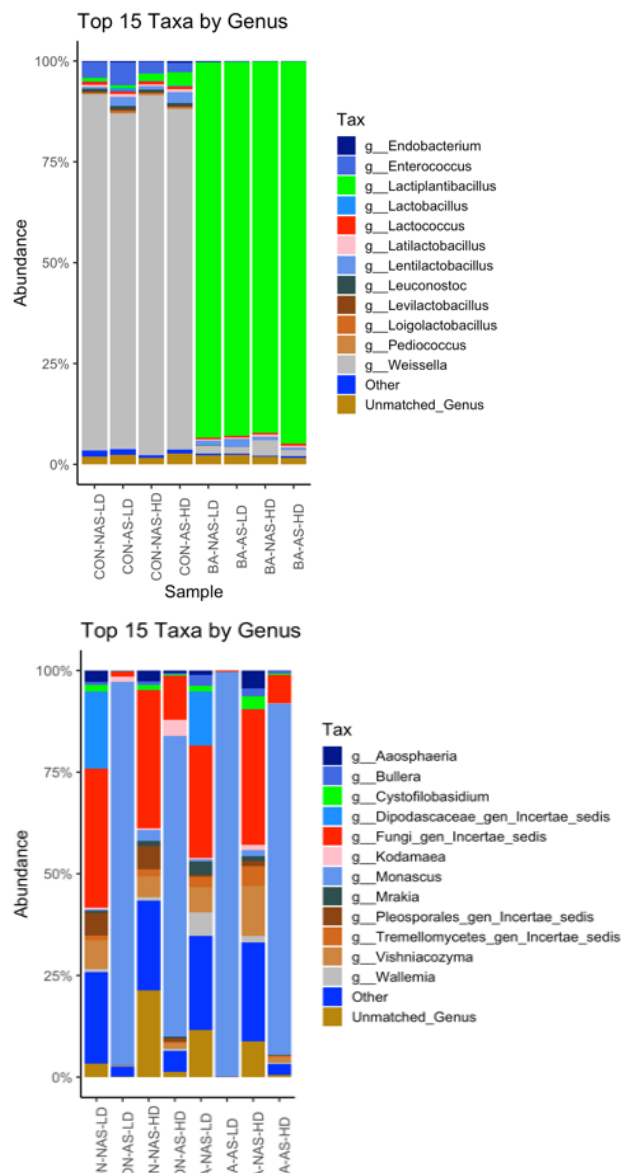
Fermentation characteristics are presented in Table 1. There were no 3-way interactions between INO, AIR, and D, but multiple main effects and 2-way interactions were observed. In general, AS negatively impacted the silage as evidenced by higher mold and lower aerobic stability; mold, pH, and reduced organic acids were more pronounced at LD. The BA treatment improved silage fermentation as evidenced by increased DM, decreased pH, and increased lactic acid, but did not increase aerobic stability. The BA treatment also increased 1,2-propeniol and water-soluble carbohydrates, while decreasing butyric acid (present at very low concentration) and NH₃-N (data not shown).

Table 1. Effect of treatment on selected silage characteristics.

Treatment	DM, %	pH	LAB, log ₁₀ cfu/g	Molds, log ₁₀ cfu/g	Lactic acid, % DM	Acetic acid, % DM	Aerobic stability, h
CON-NAS-LD	38.3	4.5	5.4	<2.0	6.2	2.2	148
BA-NAS-LD	40.5	4.3	5.1	<2.0	6.9	1.8	158
CON-AS-LD	37.7	4.6	6.6	4.3	5.9	2.3	60
BA-AS-LD	39.4	4.3	6.7	4.3	6.9	1.6	69
CON-NAS-HD	37.3	4.5	5.5	<2.0	6.6	2.4	143

Alpha diversity (Shannon index and observed species) was affected by treatments (Kruskal-Wallis, $P < 0.05$) with more diverse bacterial and fungal communities observed in CON vs. BA and NAS vs. AS, respectively (data not shown). *Lactiplantibacillus* dominated BA treated silage (relative abundance [RA] 93%) whereas *Weissella* dominated CON treated silage (RA 87%; Figure 1). Bacterial communities were not affected by AIR and D. Fungal communities were affected by AIR, with *Monascus* (RA 89%) dominating in AS conditions. Fungal communities were not affected by INO or D.

Figure 1. Effect of treatment on bacterial and fungal communities.



Conclusions

The AS treatment, especially at LD, reduced aerobic stability. The BA treatment decreased pH and increased lactic acid, but did not affect aerobic stability. For bacteria, *Lactiplantibacillus* dominated the BA treatment, even with the AS and LD challenges. For fungi, *Monascus* dominated under AS conditions and may have driven the reduced aerobic stability.

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CONFOUNDING EFFECT OF IN-FIELD VARIABILITY ON CORN SILAGE FEEDING TRIALS

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Keywords: corn silage, micro-environment, quality variability, feeding trial design

Introduction

Forage, and especially corn silage, has far more quality variability than commodity feeds. Experimental design recommendations often fail to acknowledge that corn silage, as well as other forages, are not homogeneous (Uden, 2012). Corn silage fractions such as starch and fiber independently fluctuate in relative amount and digestibility. Unlike commodity feeds and grains, corn silage experiences little or no comingling to dilute in-field micro-environment variation from time of harvest until consumed by livestock. In-field variability impacting quality attributes is far more substantial than historically assumed. This variation is primarily due to micro-environments in which the plants grow. The resulting inconsistencies in quality create a confounding effect severely limiting the ability to produce a control corn silage for feeding trials studying specific quality parameters. These influences of growing environment variability have been underappreciated in the scientific literature. Uncontrolled variables in corn silage quality should be acknowledged and addressed in feeding trial protocols and analyses. Failure to account for the confounding corn silage quality variability has resulted in erroneous conclusions in numerous otherwise well-designed, peer-reviewed feeding trials. Among the more common examples of these circumstances are trials evaluating starch digestibility between corn hybrid traits that have notable differences in starch content (unrelated to starch digestibility traits) between treatments which go unaccounted, thus confounding animal performance data interpretation.

Materials and methods

Genetic, environmental, and management influences converge to create unique yield and quality observations in corn silage. Many of these influencing factors are understood in the plant science field. These typically represent rather complex physiological and practical mechanisms in and around the corn plant. For instance, starch content of corn silage is approximal to the amount of grain yield relative to vegetative biomass yield. Vegetative biomass is most influenced by growth parameters early in the growing season. Optimal growing conditions (light, temperature, water, nutrients) during vegetative growth is likely to result in a larger, more robust, higher biomass yield plant structure. However, if that same plant, then experiences a stressed environment, possibly drought or foliar disease during grain fill, starch yield will be reduced thereby resulting in a relatively low analysis of percent starch. The opposite during a more stressful early growing season and highly favorable grain fill period may also be true. These opposing conditions can occur within a single field as soil type, soil-structure, fertility, drainage, crop residue, soil preparation, pests, and countless other factors frequently vary within a relatively small area of a seemingly uniform field. Similar variation exists in all primary feed quality attributes of interest. Quality factors with in-field variability that are especially prone to being overlooked include foliar diseases that may reduce fiber digestion and/or generate toxins and varying epiphytic populations that influence silage fermentation pathways and efficiency.

Data from numerous fields trials and hybrid performance experiments were evaluated for variability across diverse geographies and within individual fields. Yield, dry matter content, starch content, fiber digestibility, and pre-ensiled starch digestibility were the areas of focus for these analyses.

An analysis was conducted on actual field data to demonstrate the difficulty in producing two separate silage inventories of equal quality for a hypothetical feeding experiment evaluating a single quality attribute.

The effectiveness of using genetic isolines for the elimination of genetic variation when studying a particular genetic trait was also investigated.

Results

Variation in corn silage quality attributes (%DM, %starch, %NDFD24/NDFD30, %IVSD7/ISSD7) tends to diminish when reducing geographic scope. However, the variation within a single hybrid, within a single field remains relatively large. In-field variability can be as significant as the variation within the same hybrid across a geography representing many diverse fields and growing environments. Each quality variable acts relatively independent of other quality attributes.

Case study and hypothetical analysis of forage harvester on-board NIRS yield, %DM, and %starch grid data demonstrated that practical efforts to minimize variation between silage treatments for feeding trial purposes may not be as effective as commonly assumed.

Genetic isolines of corn hybrids are often used for evaluating the merits of a particular genetic trait in the improvement of feed quality. While using a common germplasm with and without the trait of interest is a means of reducing sources of error and variation, comparisons of varying trait packages of the same isoline reveal that genetic trait insertion, whether via back-cross breeding or cellular modification, often alters other plant characteristics. Plant health and relative maturity are examples of frequent unintended phenotype changes that influence feed quality parameters. Unique germplasms often respond differently to insertion of the same genetic trait.

Conclusions

Variability of individual quality metrics within a single, generally uniform field can be reduced but not eliminated. This variation is enough to confound and distort feeding trials that are specifically focused on a single quality attribute, by the inability to control other quality variables. Efforts should be made to thoroughly assess and document the growing environment in any field being considered for producing corn silage for feeding trials. The research community should create consensus on how to address uncontrolled variables in corn silage (and other forages) that are likely to confound an experiment. For instance, should experimental diets be made “fermentable starch neutral” like other dietary nutrients, such as nitrogen, are held equal in experimental design? At a minimum, investigators should report efforts and actions to reduce and address in-field quality variation, including growing season and crop agronomic observations.

Table 1. In-Field Quality Influencers and Variability Reduction Practices to Consider (incomplete listings)

Quality attribute	Influencing factors	Reduction practices
Dry matter content (%DM)	Plant health, harvest timing, moisture stress	Field selection; assure ample fertility via current grid soil testing and variable rate fertilizer application; verify uniform seed bed preparation and planter unit functionality; alternating treatment strips in field; plant same date; harvest same date, time and maturity (as determined by plant observation, not by company ratings); strip orientation to sub-surface drainage; irrigation; foliar fungicides; in-season nitrogen management; use a highly dominant silage inoculant to standardize fermentation; scout field throughout growing season to document visual signs of variability (consider using Normalized Difference Vegetation Index imagery, NDVI)
Starch content	Moisture stress, K uptake & timing, N uptake & timing, plant health, harvest kernel maturity, harvest cut height	
Fiber digestibility (%NDFD)	Plant health, harvest timing, K uptake & timing, N uptake & timing, moisture stress	
Starch digestibility	N uptake & timing, harvest kernel maturity, kernel processing, relative dry matter of stover to grain	
Protein content	N uptake & timing, moisture stress, plant health	

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Integration of Foundational Models on Autonomous Drones for Silage Inventory and Storage Quality Assessment

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Keywords: Drones, Farm Management, Foundational Models, Silage, Precision Agriculture

Introduction

Agricultural robots are recognized as transformative tools to address critical challenges in modern farming, such as precision agriculture, labor shortages, and the sustainable practices [1-4]. Among these, drones equipped with advanced sensors and artificial intelligence offer immense potential to revolutionize agricultural inspections. They can monitor crop health, assess animal welfare, and evaluate infrastructure conditions with unparalleled efficiency [5]. However, the deployment of such technology in agricultural settings faces two significant barriers: a scarcity of domain-specific datasets for training AI models and the complexity of drone operation, which makes them less accessible to farmers. To overcome these challenges, we propose a novel drone-based system that integrates Large Language Models (LLMs) with advanced visual analysis capabilities and classical photogrammetry to serve as an intelligent assistant for farm management. LLMs have a proven record of showing promising results in drones to address data scarcity [6-8] and in agricultural inspections [9-11]. Our primary objective is to enable drones to evaluate silage inventory automatically, irrespective of silo type, while identifying and reporting issues in silage storage. By harnessing the power of LLMs, the system will process natural language inputs both text and voice from farmers, translating them into actionable inspection tasks. This approach simplifies interaction and empowers farmers to efficiently manage inspections without requiring technical expertise. Equipped with RGB cameras, the drone system will conduct comprehensive assessments of silage bunkers, tower silos, and other storage structures. It identifies inventory levels, detect anomalies (e.g., spalling, cracks, improper sealing, or spoilage) and provide detailed analysis to optimize storage conditions and minimize feed loss. The ability to adapt to various silage management types and operational contexts is a key feature of the system, ensuring its broad applicability across diverse agricultural settings.

Methodology for Developing the Model

The system automates drone control, evaluates silage inventory, and identifies silage storage issues through software development, model training, and hardware integration.

Graphical User Interface and Task Input: Graphical user interface (GUI) was developed for task input via text or voice using OpenAI's Whisper. The GUI converts inputs into structured prompts using prompt engineering and zero-shot Chain of Thoughts. The prompt is then sent to an LLM (e.g. DeepSeek [12]) which will generate a step-by-step instruction to perform the actionable task. This instruction is sent to an NVIDIA Jetson Orin on the drone, specifying objectives like inventory assessment or anomaly detection.

Automated Drone and Data Collection: A flight control algorithm interprets task prompts to generate an optimized inspection path for DJI M30 drone using ROSA (ROS Agent) [13]. The drone captures images at set intervals, with altitude, camera angle, and field of view dynamically adjusted to cover the silages. Calibration accounts for lighting and silo characteristics to ensure accurate data collection.

Inventory Evaluation: Silage inventory is estimated using a computer vision model (MASt3R [14]) that applies photogrammetry to reconstruct a 3D representation of silage bunker, bale and piles. The volume calculated by integrating the reconstructed surface and silage density (user provided) generates inventory analysis (scale in dry matter (DM)). Variations in pile consistency and distribution are reported.

Silage Issue Detection: Silage issues are identified using Visual Language Model (VLM) LLaVA [15], which process images and interpret task prompts. The system detects structural problems (cracks, spalling), sealing issues (gaps, loose covers), and spoilage (discoloration, mold). Anomalies are annotated visually and paired with descriptive text, providing real-time feedback.

Data Processing and Reporting: Images and analyses are transmitted to operator's computer. A summarization combines observations into a structured report highlighting inventory levels, anomalies, and recommendations. The report is in text and audio formats to provide a user-friendly interface.

Integration and Testing: The system is tested across different silo types, bunkers and environmental conditions, validating inventory accuracy, anomaly detection precision, and drone control algorithms.

Results

The methodology has been evaluated through simulated environments (Gazebo) and synthetic datasets to validate its core functionalities. The GUI successfully converts user inputs into structured prompts, and the flight control algorithm generates optimized paths for drone inspections. Preliminary tests using synthetic silage images demonstrate the system's ability to detect anomalies such as rust, structural damage, cracks and mold. VLM was able to describe the state of the bunker and silo based on the images with 80% and 90% accuracy, respectively. Example outputs from the VLM and generated summaries highlight the system's potential for delivering actionable insights: 1) For bunkers, the analysis described that the bunker images showed improper piling, presence of debris and inappropriate location with a recommendation to use airtight materials and cleanliness to avoid spoilage and contamination; 2) For silos, structural problems (cracks, leaning structures and rust) were identified and suggested the need for maintenance and repairs to avoid degrading the silage quality and to monitor moisture and temperature. Field tests scheduled for June 2025 and validate the system under real-world conditions, with a focus on accuracy, usability, and adaptability.

Conclusion

This project demonstrates the potential of integrating drones, natural language processing, and visual analysis to automate silage inventory evaluation and issue detection. Preliminary results highlight the system's ability to process tasks, perform inspections, and identify anomalies effectively. Field testing in June 2025 will validate its real-world performance, guiding further optimization. This approach aims to enhance silage management, reduce waste, and support sustainable farming practices.

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Adapting the CSM-CERES-Maize model for ability to simulate maize silage production and quality

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Keywords: Crop Modeling, Silage Production, Nutritive Value, Milk-line, Dry Matter Concentration

Introduction

Maize silage production provides important energy and nutritional contributions to dairy and beef industries. There are many process-based crop simulation models for maize, but very few for silage production. Models for maize silage would benefit from additional abilities such as predicting fresh weight production, dry matter concentration over time to select optimum harvest, total digestible energy, and crude protein. Our objective was to modify the code of the CSM-CERES-Maize model to create a model capable of simulating silage fresh weight production, milk line progression, percent dry matter of total crop, along with quality aspects (starch, crude protein, digestible organic matter) in response to weather, soil and cultivar.

Materials and methods

Code of the CERES-Maize model in DSSAT (Hoogenboom et al., 2019) was modified to predict milk line, dry matter concentration of vegetative, cob, grain, total crop, and starch, and digestible organic matter.

Milk line is commonly used as indicator for optimal plant harvest moisture (Wiersma et al., 1993; Havilah et al., 1995). The 50% kernel milk line (half-way from kernel crown to tip) occurs about 13 days before physiological (black-layer) maturity (Wiersma et al., 1993). Data from Wisconsin and Spain were used to calibrate dry matter concentration (DMC) versus milk line, where zero milk line occurs at the soft dough stage which is 180 growing degree days (GDD) after the beginning of grain growth. Thereafter, milk line is simulated to increase with GDD. The DMC of vegetative, cob, grain, and total are simulated as a function of GDD and milk line, to allow predicting the optimum DMC of total crop for harvesting.

Digestible organic matter for dairy is computed based on starch equivalent which is a function of grain growth, milk line progression, and target starch concentration parameters of vegetative and grain (Khan et al., 2015). CERES-Maize already predicts and outputs crude protein of vegetative and grain.

Weather and soil data were input to the model. Data files of yield and nutritive values were created for comparison to model outputs. Hybrid genetic parameters were calibrated to attain the observed life cycles. Then parameters related to milk line, DMC, and fresh weight production were calibrated and evaluated with data on 3 hybrids at 4 sites in Asturias, Spain over 3 years. Model simulations were evaluated with data on 6 hybrids sown in March and July during 6 years (2018-2023) at Citra, Florida.

Results

The progression of milk line (Figure 1A) and grain DMC (Figure 1B) over time was successfully calibrated with data from Wisconsin (Wiersma et al., 1993) and Spain. The crop DMC over time and total crop fresh weight over time were evaluated with data on 3 hybrids over 4 sites and 3 seasons in Asturias, Spain (Oliveira et al., 2023). Model simulations were evaluated with data on 6 hybrids with spring and summer sowing dates over 6 years (2018-2023) at Citra, Florida where the model showed good prediction of dry matter yield, DMC%, crude protein and starch%, with average nRMSE of 15-18% (Figure 2).

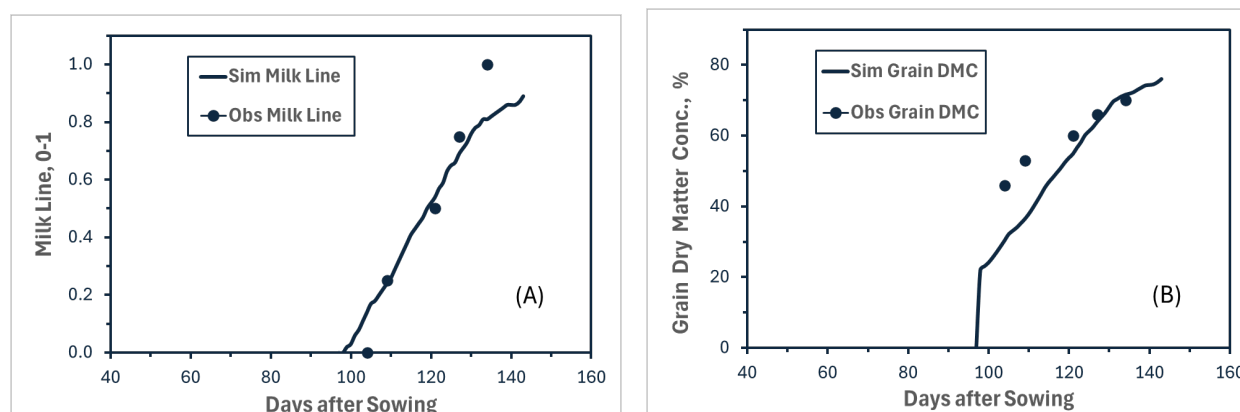


Figure 1. Milk line progression over time (A) and grain dry matter concentration over time (B), observed and simulated (after calibration) for maize cultivar grown in 1990 in Wisconsin (Wiersma et al., 1993).

Figure 2. Observed (points) and predicted (lines) DM concentration (A), yield (B), starch concentration (C), and crude protein concentration (D) along years for spring and summer sowing dates in Citra, Florida.

Conclusions

The Maize-Silage model will be in a future release of DSSAT and has potential use as a tool to evaluate production and nutritive value as affected by N fertilization, irrigation, sowing date, and harvest timing.

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Amino Acids in Lucerne Silage: 2. Effects of Inoculation

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Keywords: lucerne, inoculation, amino acids

Introduction

Silage quality is strongly influenced by the degree of protein degradation that occurs after harvest and during fermentation (Albrecht and Muck, 1991). Lucerne has a low sugar content and high buffering capacity making the forage more difficult to ensile effectively. Achieving a low silage pH rapidly will reduce the rate of proteolysis, and inoculation with a homofermentative bacteria can achieve this. Jones *et al.* (1992) reported that inoculation reduced ammonia-N, free amino acids and non-protein nitrogen content in silage. Here, we investigate the role of silage inoculant on the amino acid profile of lucerne silage.

Materials and methods

A lucerne (*Medicago sativa*) crop was cut on 16 August 2022 (third harvest, 5 weeks growth) using a hand operated reciprocating blade mower. The forage was spread across the ground and allowed to wilt for 24 h. The forage was passed through a stationary forage harvester and cut to 12mm theoretical chop length. Forage (c. 1260 g) was ensiled for 96 days in 2 L glass silos either untreated or with a commercially available homofermentative *L. plantarum* inoculant NCIMB 40027 (Ecosyl 100, Volac Ltd, Royston, Herts., UK; 1×10^6 CFU g⁻¹). Samples of forage as mown and as ensiled and the resultant silages were freeze dried prior to determination of ash, water soluble carbohydrate (WSC), neutral-detergent fibre (NDF) nitrogen (N) and buffer soluble N content. Ammonia-N, pH, lactate, volatile fatty acid (VFA) and ethanol content was determined on frozen silage samples. Amino acid (AA) concentrations were determined in freeze-dried samples by Sciantec Analytical Services Ltd (Berkshire, UK) using ion exchange chromatography.

Results

Inoculation resulted in an improved fermentation with significantly lower silage pH, acetate, ethanol and ammonia-N concentrations, and a higher lactate concentration (Table 1). N solubility increased due to ensilage but there was no difference in N solubility due to inoculation. Inoculation resulted in a silage with higher concentrations of total and essential amino acids ($P < 0.001$). Although inoculation resulted in silage with a higher concentration of total free amino acids ($P < 0.001$), the concentration of free essential amino acids was lower in the inoculation silage ($P < 0.007$) (Table 1). When free AAs were determined as a percentage of total AAs, inoculated silage had lower free amino acids (57.0 v 61.6; s.e.m. 0.59; $P = 0.005$) and free essential AAs (33.4 v 41.5; s.e.m. 0.44; $P < 0.001$) compared to untreated silage. Inoculation also reduced the free essential AAs when determined as a percentage of the total essential AAs (66.1 v 76.2; s.e.m. 0.78; $P < 0.001$) when compared to untreated silage.

Effects of inoculation on total concentrations (g/kg DM) of the individual AA's are demonstrated in the biplot in Fig 1. Principal component 1 accounted for 74% of the total variation with clear separation of the treatments on this axis. Based on analysis of variance of individual AA's (data not shown), the essential AA's threonine, histidine, lysine and arginine as well as non-essential aspartic and tyrosine were higher ($P < 0.05$) in inoculated silage, whilst the essential AA's iso-leucine and leucine and non-essential glycine, alanine and proline were lower ($P < 0.05$) in inoculated silage (Fig 1). In the free form, percentage of the essential AA's methionine, valine, iso-leucine, leucine, phenylalanine, histidine as well as the non-essential cystine, glutamic, glycine, alanine, and proline were lower ($P < 0.01$) in inoculated silage, whilst the essential AA's threonine, lysine and arginine and non-essential aspartic, serine and tyrosine were higher ($P < 0.05$) in inoculated silage (data not shown).

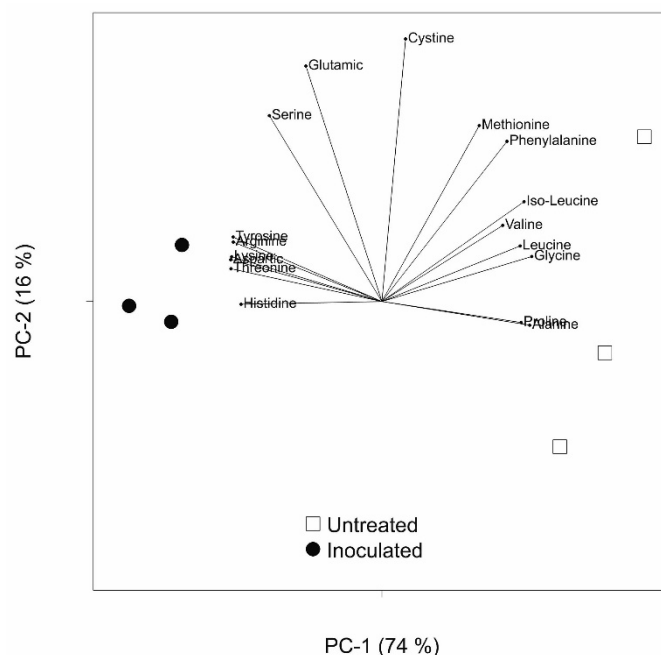
Conclusions

Overall, the use of a silage inoculant resulted in a silage with higher concentrations of total and essential amino acids of lucerne silage, significantly increasing threonine, histidine, lysine and arginine essential amino acids.

Table 1. Chemical Composition of lucerne forage at mowing, ensiling and after ensiling for 96 days,

	At mowing*		At ensiling*		Silage**			
	Mean	s.e.m.	Mean	s.e.m.	Untreated	Inoculated	s.e.m.	Prob
FD DM (g/kg)			330.6	2.64	317.4	325.1	2.51	0.096
Ash (g/kg DM)	98.5	2.65	97.2	2.40	103.5	101.6	2.96	0.681
WSC (g/kg DM)	60.9	2.97	70.4	1.46	18.0	18.0	0.56	0.952
NDF (g/kg DM)	406.0	16.45	406.6	9.36	380.7	365.0	12.86	0.436
pH	-	-	-	-	5.45	4.40	0.043	<0.001
Lactate (g/kg DM)	-	-	-	-	41.2	79.6	1.04	<0.001
Acetate (g/kg DM)	-	-	-	-	24.4	18.4	0.50	0.001
Ethanol (g/kg DM)	-	-	-	-	5.83	2.85	0.314	0.003
Lactate (% TFA)	-	-	-	-	60.0	77.6	0.74	<0.001
Total N (g/kg DM)	30.4	0.51	28.9	0.73	32.8	32.1	0.35	0.237
Soluble N (g/kg N)	438.2	5.89	438.9	11.17	839.3	807.5	13.75	0.177
Ammonia (g/kg N)	-	-	-	-	224.2	92.5	4.83	<0.001
Amino acids (g/kg DM)								
Total	170.1 ¹	-	-	-	134.8	165.4	1.44	<0.001
Total essential	83.3 ¹	-	-	-	73.4	83.6	0.73	<0.001
Total Free	8.17 ¹	-	-	-	83.1	94.2	0.19	<0.001
Free essential	2.47 ¹	-	-	-	56.0	55.2	0.10	0.007

* 3 sub samples; ** 3 replicates per treatment; TFA - total fermentation acids; ¹ single determination

**Figure 1.** Principal component (PC) biplot of total concentrations (g/kg DM) of amino acids in silages

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Impact of Ecocool Silage Inoculant on Corn Silage Digestibility and Mycotoxin Contamination After Air Exposure

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Keywords: aerobic spoilage, corn silage, mycotoxins, NDF digestibility, Ecocool

Introduction

Corn silage is the primary forage for dairy and beef cattle, valued for its palatability, digestibility, and high energy content. However, it exhibits high susceptibility to aerobic spoilage particularly during the feed-out phase. Aerobic deterioration of silage can lead to significant losses, with up to 20% of dry matter (DM) potentially lost due to the activity of undesirable yeasts and molds (Borreani *et al.*, 2018). These losses also diminish the nutritional value of the feed and can negatively impact intake and compromise animal health through production of mycotoxins. Therefore, preserving silage quality is crucial for maximizing farm efficiency and sustainability. The Ecocool silage inoculant contains *Lactiplantibacillus plantarum* strain MTD/1 and *Lentilactobacillus buchneri* strain PJB/1. Much of the research using such combination inoculants (containing both homofermentative and heterofermentative strains) focusses primarily on aerobic stability of the resultant silage. In this study, we assessed the impact of Ecocool not only on silage aerobic stability, but also on silage nutritional and hygienic quality.

Materials and methods

Field-harvested corn was transported to the laboratory for ensiling. Forage (22% DM) was either left untreated (CON) or inoculated with Ecocool (EC) at a target total LAB count of 3.0×10^5 cfu/g fresh forage. Forages were ensiled in 1.8L glass jars and stored at $20 \pm 1^\circ\text{C}$ for 46 or 95 days. Upon silo opening, each replicate was thoroughly homogenized, and representative subsamples were analyzed to determine silage quality parameters (DM, microbial populations, pH, fermentation acids/alcohols), and aerobic stability. DM of silages was determined by oven drying at 60°C until constant weight. Fermentation acids and alcohols were quantified using high-performance liquid chromatography (HPLC) coupled with a refractive index detector. After silo opening, aerobic stability was assessed at $20 \pm 1^\circ\text{C}$, by monitoring changes in temperature at 15-minute intervals using a probe attached to a data logger (Grant). Aerobic stability was defined as time needed until silage temperature reached 2°C above the ambient. Mycotoxin levels were measured in the corn pre and post ensiling, and after aerobic exposure. Mycotoxin quantification was performed using HPLC-mass spectrometry (HPLC-MS). Silage samples were analyzed for NDF digestibility using wet chemistry methods. Statistical analysis was conducted using ANOVA with Tukey's Honestly Significant Difference (HSD) post-hoc test in R (version 4.3.1.) Statistical significance was set at $\alpha = 0.05$.

Results and discussion

After 46 days of ensiling, EC-treated corn had significantly lower DM losses relative to CON (8.26 and 8.97 g/kg DM respectively; $p=0.042$). Acetic acid was significantly elevated in the EC silage (17.58 and 24.14 g/kg DM for CON and EC respectively; $p = 0.003$) resulting in a significant reduction in yeasts (5.70 and 4.22 $\log_{10}$ cfu/g for CON and EC silages respectively; $p=0.009$) and an enhanced aerobic stability (Table 1) relative to CON. By day 95 post-ensiling, EC-treated silages showed significantly higher concentrations of acetic acid (19.71 and 33.19 g/kg DM for CON and EC respectively; $p<0.001$) and 1,2-propanediol ($<\text{LOD}$ and 10.91 g/kg DM for CON and EC respectively; $p<0.001$), both key indicators of secondary fermentation driven by *L. buchneri* (Oude Elferink *et al.*, 2001). While yeasts persisted in the CON silage (2.56 $\log_{10}$ cfu/g), they were undetectable ($<2.30 \log_{10}$ cfu/g) in EC silages (diff. not significant). Testing consistently demonstrated that corn inoculated with EC exhibited significantly greater aerobic stability compared to CON regardless of fermentation time ($p = 0.002$ and $p < 0.001$ for day 46 and 95 respectively; Table 1). Furthermore, after 95 days of ensiling and 12.73 days of aerobic stability, EC-treated silages showed significantly lower aerobic stability losses than CON silages (5.93 and 12.70 g/FM respectively; $p = 0.002$). Following 95 days of fermentation and 12.73 days aerobic exposure, mold was clearly visible on the surface of CON silages, while no visible mold was present in EC silage (result not shown). Silages were therefore tested for the presence of mycotoxins. Of the 18 mycotoxins analyzed, four were detected in the silages. Low levels of zearalenone (ZON), and high levels of deoxynivalenol (DON) were detected in both EC and CON silage (Table 2), although notably, high DON levels (10,229 ppb) were also present in the corn prior to ensiling. Mycophenolic acid (MPA) and roquefortine C (RoqC) were detected in CON silages following aerobic exposure (table 2), but not at the time of silo opening ($< \text{LOD}$ and 4ppb for RoqC and MPA respectively) indicating that contamination with these mycotoxins resulted from fungal growth during exposure to air. No MPA and RoqC were detected in EC-treated silages at silo opening. After aerobic exposure levels of MPA and RoqC in EC silages were significantly lower relative to CON (Table 2; $p < 0.001$ and $p < 0.001$ for MPA and RoqC respectively), representing approximately 98% reduction.

Table 1. Aerobic stability of corn silage after 46- and 95-days fermentation period $\dagger$.

Treatment	Aerobic Stability (d)	
	46d fermentation	95d fermentation
CON	1.49 ^b (± 0.35)	8.03 ^b (± 0.51)
EC	3.50 ^a (± 0.28)	12.73 ^a (± 0.16)

$\dagger$ Means within a column not followed by the same letter are statistically different ($P < 0.05$).

Table 2. Mycotoxins detected in corn silage after 95 days of ensiling and 12.73 days of aerobic stress †.

Treatment	DON	ZON	MPA	RoqC
	ppb*			
CON	17,871 ^a (±1,134)	16 ^a (±28)	5,233 ^a (±1,017)	8,726 ^a (±1,455)
EC	17,670 ^a (±526)	18 ^a (±31)	127 ^b (±219)	197 ^b (±320)

† Means within a column not followed by the same

letter are statistically different (P < 0.05). *Corrected to 88% DM.

EC treated silages fermented for 95 days and exposed to air for 12.73 days showed significantly higher NDF digestibility relative to untreated silages with the greatest difference seen at 30 h (p = 0.010; Table 3). Oba and Allen (1999) reported that improving forage NDF digestibility by 1% can lead to a 0.17kg increase in DMI and a 0.25kg increase in 4% fat-corrected milk yield. These results suggest that applying Ecocool has the potential to not only enhance the nutritive value of the silage but also significantly improve animal performance.

Table 3. NDF digestibility of corn silage after 95 days of ensiling and 12.73 days of aerobic stress †.

Treatment	% NDF digestibility					
	12h	24h	30h	48h	120h	240h
CON	16.67 ^b (±0.31)	29.70 ^b (±0.56)	34.87 ^b (±0.67)	46.50 ^b (±0.89)	63.50 ^b (±1.13)	66.67 ^a (±1.15)
EC	18.93 ^a (±0.98)	33.50 ^a (±1.39)	39.07 ^a (±1.44)	50.90 ^a (±1.30)	65.87 ^a (±0.21)	67.90 ^a (±0.50)

† Means within a column not followed by the same letter are statistically different (P < 0.05).

Conclusions

Applying Ecocool silage inoculant significantly improved the aerobic stability of corn silage by generation of acetic acid and suppression of yeasts and molds during exposure to air. This resulted in enhanced hygienic quality of the silage by inhibiting the production of mycotoxins and significantly improving silage NDF digestibility.

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Effect of COMBINATIONS OF LACTIC ACID BACTERIA on the FERMENTATION of sugarcane silage

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Keywords: acetic acid, ethanol, *Lentilactobacillus buchneri*, yeasts, *Lactococcus lactis*

Introduction

In tropical climate regions, sugarcane (*Saccharum officinarum* L.) is an important roughage source, with a high dry matter yield potential (40 to 50 t DM ha⁻¹) and greater nutritional value during the dry season. However, ensiling sugarcane often increases crop longevity through more uniform harvests and better agronomic management, decreases the risk of lodging, and eliminates daily harvests, which optimizes logistics and proper management. Despite its favorable characteristics for lactic fermentation, such as adequate DM content, low buffering capacity, and high soluble sugar content; sugarcane silage preserved by natural fermentation suffers significant DM loss (≥ 200 g kg⁻¹ DM), due to the conversion of soluble sugars into ethanol and CO₂ by yeast metabolism (Pedroso et al., 2005). Hence, the use of silage inoculants based on heterofermentative lactic acid bacteria capable of producing acetic acid is a possible strategy to reduce yeast activity both during storage and in the feeding phase, minimizing nutrient losses. Therefore, the aim of this study is to examine the effect of SiloSolve® FC and SiloSolve® AS on the fermentation profile and dry matter loss of sugarcane silage at two storage periods.

Materials and methods

Sugarcane was harvested after 14 months of regrowth at approximately 18° brix (DM = 30.3% FM, lactic acid bacteria count = 7.26 log CFU g FM⁻¹, yeast count = 4.63 log CFU g FM⁻¹) and chopped in a stationary forage chopper (10 mm theoretical length of cut). Chopped sugarcane was divided into 30 piles to receive one of three treatments (10 piles per treatment): 1) control (without inoculant; **CON**); 2) SiloSolve® AS containing *Lentilactobacillus buchneri* DSM22501 (7.5×10^4 CFU g FM⁻¹), *Enterococcus lactis* DSM22502 (4.5×10^4 CFU g FM⁻¹) and *Lactiplantibacillus plantarum* DSM16568 (3.0×10^4 CFU g FM⁻¹) (**AS**); 3) SiloSolve® FC containing *Lactococcus lactis* DSM11037 (7.5×10^4 CFU g FM⁻¹) and *Lentilactobacillus buchneri* DSM22501 (7.5×10^4 CFU g FM⁻¹) (**FC**). Inoculants were diluted in distilled water and applied using hand sprayers (10 mL kg⁻¹ FM). The control was receiving the same amount of distilled water. After manual homogenization, ~6.0 kg of treated forage was ensiled in 7.2-L plastic buckets (experimental silos) with lid and joints wrapped with six layers of self-adhesive tape. The silos were stored at room temperature (14 to 34 °C). Five experimental silos per treatment were opened after **14 d** of storage, whereas the remaining were stored for **63 d**. At opening, silages were analyzed for microbial counts, pH, and fermentation end-products. Silage DM was corrected for losses of volatile compounds during oven drying and used to calculate fermentative losses. Data were analyzed using the proc MIXED of SAS as a completely randomized design with a factorial 4 × 2 arrangement (5 replicates/treatment). Means were compared by Tukey test.

Results

Sugarcane ensiled without inoculant (CON) had a high concentration of ethanol and as expected, a high DM loss during fermentation, especially for the longer storage period. However, sugarcane silages inoculated with AS or FC expressed a markedly decreasing ethanol formation and DM loss during storage, with a greater benefit of FC compared with AS (Table 1). In this trial, both AS and FC were capable of increasing the concentration of acetic acid at 14 and 63 d of storage, indicating a greater heterofermentative activity in inoculated silages since the onset of fermentation. The concentration of 1,2-propanediol was increased for AS at 63 d and for FC at 14 and 63 d of storage, suggesting that the acetic acid was partially derived from lactic acid conversion to acetic acid and 1,2-propanediol (Oude Elferink et al., 2001) and that FC acted faster than AS to curtail sugarcane silage loss. Acetic acid is an antifungal compound, capable of inhibiting yeast metabolism and ethanol production, which in turn decreases CO₂ formation and DM loss.

Table 1. Fermentation profile and dry matter loss of sugarcane silage stored for 14 or 63 d

Item	Storage, d	Treatment ¹			SEM ²	P-value ³		
		CON	AS	FC		T	S	T × S
DM ⁴ , %FM	15	28.0c	29.1b	30.2a	0.18	<0.01	<0.01	<0.01
	60	26.5d	29.3b	30.2a				
Lactic acid bacteria, log CFU g FM ⁻¹	15	6.81c	8.62a	7.88b	0.068	<0.01	<0.01	<0.01
	60	5.93d	5.64d	4.75e				
Yeast, log CFU g FM ⁻¹	15	3.73ab	3.59b	3.55b	0.180	0.16	<0.01	0.47
	60	4.45a	4.38a	3.93ab				
pH	15	3.22d	3.13e	3.15e	0.005	<0.01	<0.01	<0.01
	60	3.45a	3.28c	3.31b				
Lactic acid, %DM	15	4.92cd	6.72ab	4.22d	0.260	<0.01	<0.01	0.08
	60	5.86abc	6.94a	5.66bc				
Acetic acid, %DM	15	1.47d	2.91c	4.14a	0.079	<0.01	<0.01	0.08
	60	1.77d	3.49b	4.36a				
Ethanol, %DM	15	9.24b	4.49c	1.09e	0.145	<0.01	<0.01	<0.01
	60	12.2a	5.07c	2.12d				
(R,R/S,S)-2,3-Butanediol, %DM	15	0.037cd	0.072b	0.067bc	0.008	<0.01	<0.01	<0.01
	60	0.032d	0.171a	0.072b				
(R,S)-2,3-Butanediol, %DM	15	0.249b	0.173c	0.249b	0.014	<0.01	<0.01	<0.01
	60	0.3706a	0.176c	0.295b				
Sum 2,3-Butanediol isomers, %DM	15	0.286cd	0.245d	0.316bc	0.015	0.01	<0.01	0.09
	60	0.403a	0.347abc	0.368ab				
1,2-Propanediol, mg kg DM ⁻¹	15	367c	273c	1786a	79.2	<0.01	<0.01	<0.01
	60	289c	924b	1921a				
DM loss, %DM	15	9.89b	5.19cd	2.12e	0.633	<0.01	<0.01	<0.01
	60	15.9a	5.53c	2.60de				

¹CON: control (without inoculant), AS: SiloSolve AS; FC: SiloSolve AS. ²Standard error of the mean. ³T: effect of inoculant, S: effect of storage period, T×S: interaction between inoculant and storage period. ⁴Dry matter corrected for losses of volatile compounds during oven drying. a,b,c,d Tukey test ($\alpha = 0.05$).

Conclusions

Inoculating sugarcane silage with SiloSolve® AS and SiloSolve® FC were effective in inhibiting yeast metabolism and mitigating dry matter loss during fermentation, resulting in sugarcane silages with higher nutritional value.

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Greenhouse Gases During Ensiling and Feed Out Phase of Corn Silages

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Keywords: corn silage, greenhouse gases, inoculants, feed-out phase

Introduction

Worldwide, there is an urgent need to reduce greenhouse gases in animal production. Concerning ruminants, the focus has been on emissions from the rumen and intestinal tract. However, during the fermentation, stabilization and feed out phases of corn silages, several chemical processes occur during which O₂ is consumed and CO₂ is produced. The exact amount of greenhouse gases emitted from silage is unknown. During the ensiling and feed out process, losses can be extensive, but those losses are usually estimated based on the expected dry matter losses (Hafner et al., 2012) or on the feeding value of the silage (Athmanathan et al., 2015). Losses may be reduced by inoculants, as inoculants reduce weight and quality losses. To test whether the use of inoculants is a useful tool to reduce greenhouse gas emissions, an experiment was performed in the respiration chambers of Wageningen UR. In animal science, open-circuit respiration chambers are used to measure gas exchange and to calculate heat production of animals. The respiration chambers at Wageningen UR are size adaptable and ambient temperature, humidity and ventilation rate can be controlled independently. This also makes them suitable to determine gaseous losses in non-animal material, such as (lab scale) silages. The goal of the experiment was to measure actual gaseous losses during ensiling and feed out of corn silage, with or without the use of inoculants.

Materials and methods

Whole crop corn (early variety LG31.218; 35% of dry matter) was harvested and cut with a precision cutter (0.9cm) on September 25th 2023. About 750 kg of chopped maize was mixed and divided into three parts of approximately 250 kg. Each part got a different treatment with water (T1) or inoculants (T2: combi E; T3: combi P – both were blends of homofermentative and heterofermentative strains), sprayed with a plant sprayer on top of the corn. Corn was mixed by hand and for each treatment, twelve 20L mini silos were filled with 16.0 kg fresh matter with help of a pneumatic press, to reach a compaction of 280 kg DM m⁻³. Subsequently, silos were closed and weighed.

During the anaerobic ensiling phase of five weeks, the mini silos were put in plastic stacking boxes (Kruizinga, Wapenfeld, The Netherlands) with closed walls. The boxes were closed with a lid, resting on a foam rubber ribbon. Six mini silos were put in one box, and two boxes were used per treatment. Air was pumped in the boxes through an attached tube at the top side of the box with a gas volume meter to measure the air flow. The gas composition of the outcoming air was analyzed by a gas analysis system. Temperature and air humidity were measured inside the box with a sensor, and every 15 minutes, CO₂, CH₄, O₂ and NH₃ concentrations were measured; N₂O was measured daily the first three days, after that weekly. After the anaerobic phase, the aerobic feed out phase was simulated. The boxes were opened, and the mini silos were weighed. For each box, all mini silos were opened and mixed. Subsequently, from all 6 silos per box, 3.5 kg was placed in a crate and thoroughly mixed together, adding up to 21 kg per crate. The crates were replaced in the original boxes, which were closed again. Temperature was measured with a sensor and gas measurements were continued during 7 days.

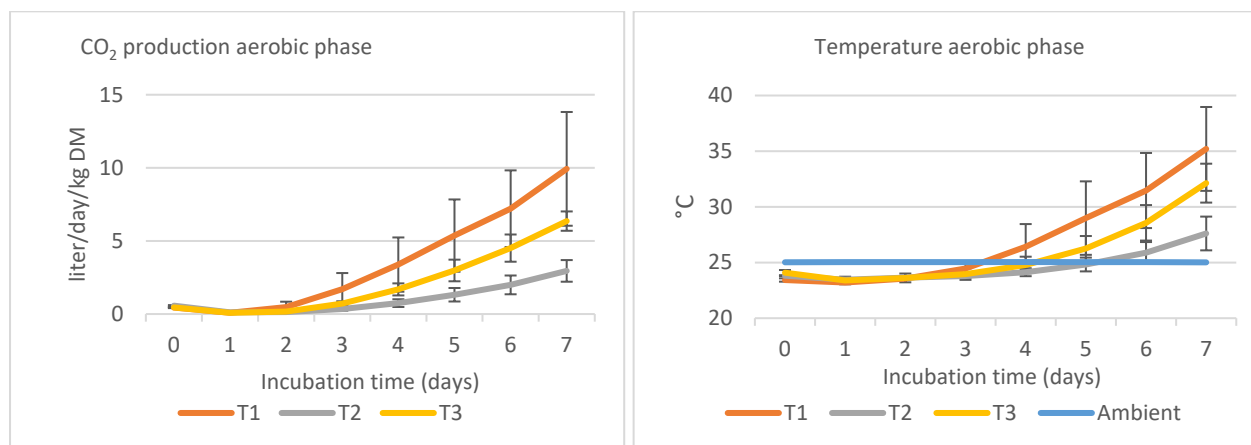
Statistical analyses were performed with Genstat (VSN International Ltd, 22nd edition) with a REML model for repeated measurements with treatment, time and their interaction as fixed effects and the subjects (boxes) as random effects.

Results

Immediately upon the start of the anaerobic ensiling phase, the CO₂ production increased steeply, with a maximum production of 6.3 L per kg DM per day for T1. For T2 and T3, CO₂ production stayed below 5 L CO₂ per kg DM per day. At the same time, the O₂ consumption increased up to 1.1 L per kg DM per day for T1 and 0.7 and 0.8 L per kg DM per day for T2 and T3. The CH₄ and N₂O production were much lower than production and consumption of CO₂ and O₂ and were up to 8 ml CH₄ and 23 ml N₂O per kg DM per day. Four days after ensiling, production of CO₂, CH₄ and N₂O were zero again, as was the consumption of O₂. NH₃ production was below the detection limit.

In the first 3 days after the feed out phase started, CO₂ production was around zero (Figure 1). However, at day 2, the CO₂ production slightly started to increase, first only for T1. The treatment T2 and T3 remained stable for a short while longer, but also here CO₂ production started to accelerate from day 3 onwards. On day 7, Treatment T1 showed a significantly higher CO₂ production and O₂ consumption than T2 (p<0.05). This was also reflected in the temperature (35.0°C on T1 at day 7 vs 27.5°C on T2).

Figure 1. The CO₂ production and the temperature of the three treatments in the feed out phase.



Differences in gas production per day also affect the total gas production during the feed out phase. For both phases, the CO₂ production and the O₂ consumption were numerically higher in T1 than in the T2 and T3, with the use of inoculants. No statistical analysis could be performed on these calculations.

Table 1. Total greenhouse gas emission in L during ensiling and feed out (7 days) for the three treatments.

		CO ₂	O ₂	CH ₄	N ₂ O	NH ₃
T1	Anaerobic	21.2	2.6	0.017	0.036	0.00
T2	Anaerobic	16.6	2.1	0.013	0.045	0.00
T3	Anaerobic	16.2	2.1	0.015	0.046	0.00
T1	Feed out	29	28	0.003	0.0014	0.00
T2	Feed out	8	4	0.015	0.0000	0.00
T3	Feed out	17	16	0.018	0.0000	0.00

Conclusions

Using inoculants during the ensiling phase will promote the conservation of corn silages without increasing CO₂ production, while it protects the silage from heating feed losses and CO₂ losses during the first days of the feed out phase. This will also coincide with a reduction of feed (quality) losses. Emissions of CH₄, NH₃ and N₂O were very low and are therefore considered neglectable. Even though the experimental design is limited to lab scale mini silos and two replicates per treatment, the potential reduction of CO₂ losses from silage using inoculants can be significant - especially during the feed out phase. Reduction may add up to 30%, to be validated in a follow up trial.

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Influence of Inoculant on Fermentation of Winter Triticale at Low Dry Matter

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Keywords: triticale, silage, inoculant, fermentation

Introduction

Winter triticale (*Triticosecale* spp.) grown and harvested at flag leaf as a high quality, high yielding, premier dairy forage, is rapidly expanding its acreage across the Northeast, Midwest, and deep into the Southern United States and Canada. The higher yields, above 2.7 tonnes of dry matter (DM)/ha, have presented some challenges drying to 35% DM ideal for ensiling while still preserving nutritional quality. Cool spring temperature and spring rains often forces farms to ensile at lower than desired DM levels. Same day ensiling is critical for preserving the very high sugar and digestible components of the forage. Farmers are presented with ensiling silage from 20 to 35% dry matter, sometimes in the same field. Using the same practices as with other similar crops, the desire is to have the same success preserving the quality when the proper inoculant is used (Muck et al., 2018).

Homofermentative inoculants improve fermentation via lactic acid production which may decrease storage losses and curtail undesirable fermentation, whereas, heterofermentative inoculants should decrease losses at feedout (Oude Elferink et al., 2001). The preferred inoculant, homofermentative or heterofermentative, has not been researched with low dry matter *Triticosecale* spp.

Materials and methods

A stand of high yielding winter triticale forage (*X tritosecale*, var. Trical Flex 719) was established at 112.1 kg of seed/ha in northern New York. Preplant fertilizer applications included 280 kg/ha 6-24-24 and 130 kg/ha 39.75-0-0. Preplant fertilizer was broadcast applied with a drop gandy. Spring top-dress fertilizer of 366 kg/ha 39.75-0-0 was applied on April 5. At flag leaf stage, the crop was harvested by cutting without conditioning with a sickle bar mower on May 24 and 6 replicate samples were collected at each 0, 2, 4, 6, 24 and 30 hours after cutting. At each time after mowing, samples for each treatment were taken, chopped at 1 inch, and treatments were applied. The treatments were water mist for control, homofermentative inoculant (MC, (*Lactiplantibacillus plantarum* (DSM16568), *Enterococcus lactis* (DSM22502) and *Lactococcus lactis* (NCIMB30117)) SiloSolve® MC, Novonesis, Lyngby, Denmark) or heterofermentative inoculant (FC, (*Lactococcus lactis* (DSM11037) and *Lentilactobacillus buchneri* (DSM22501), SiloSolve® FC, Novonesis, Lyngby, Denmark). Inoculants were applied at 150,000 CFU/g of fresh matter. Each treated sample was vacuum sealed and allowed to ferment for three weeks. Following 21 d of fermentation the samples were sent to Cumberland Valley Analytical Services, Waynesboro, PA 17268 USA. Each sample was analyzed for NIR+ which gave nutrient analyses (i.e., DM, CP, ADICP, starch, sugar, etc.), and calculated values for variables of interest to be fed to lactating dairy cows (i.e., adjusted and available protein, NEI, NEm, etc.). A fermentation analysis was run to determine the impact of inoculant on the resulting silage. All data was statistically analyzed by Statgraphics software.

Results

Wilting time (drying rate) was significantly slower than what we had experienced previously with this crop (Kilcer, personal communication). The low temperature at night slowed the impact of photosynthesis on drying the crop naturally. As temperature has a logarithmic impact on biological rate of activity, the below normal temperatures had a major impact. As shown in Table 1, even after 30 hours of drying, the crop was still well below ideal for ensiling.

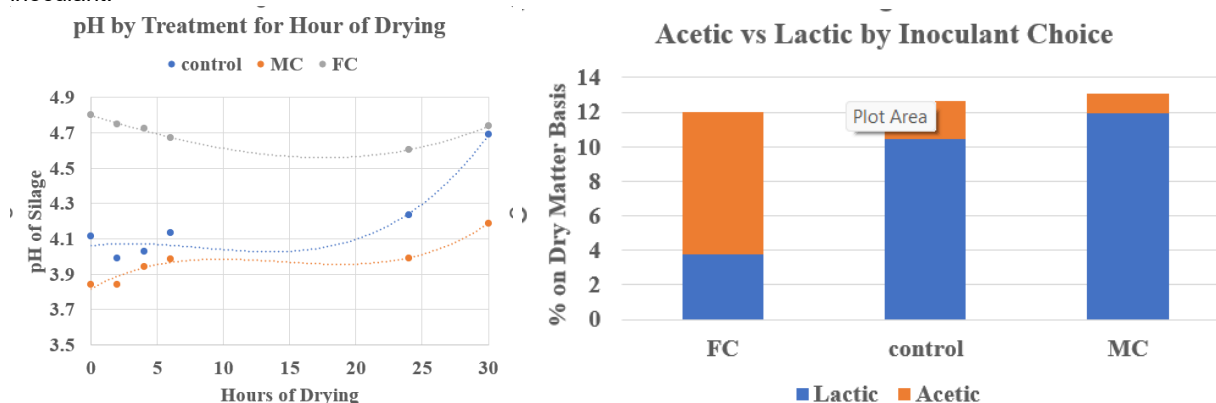
Table 1. Mean dry matter (DM%) across treatments based on wilting time.

Wilting time (h)	0	2	4	6	24	30
Triticale DM (%)	14.24 ^a	16.03 ^b	17.66 ^c	19.34 ^d	21.16 ^e	23.81 ^f

a, b, c, d, e, f Means with different superscripts differ ($P < 0.05$).

The bottom line is that this study put immense pressure on the ensiling process through suboptimal drying and consequentially lower than normal fermentable substrate levels.

Figure 1 (left): The pH of fresh Triticale by time and inoculant. Figure 2 (right): Lactic and acetic acid content (% DM basis) by inoculant.



The pH dropped for all treatments so there was sufficient substrate despite low DM conditions (Figure 1; $P < 0.05$). The final mean pH of all harvest times was significantly different between each treatment with MC having the lowest pH ($P < 0.05$; data not shown) due to higher lactic acid (Figure 2; $P < 0.05$).

Conclusions

Winter Triticale harvested at flag leaf stage is increasing across the US and in Canada. It is the earliest mechanically harvested forage but is difficult to dry when yields exceed 2.7 tonnes of DM/ha. Forages with less than ~35% DM can be challenging to ensile. Based on the results herein, the homofermentative inoculant (SiloSolve® MC) would be the logical recommendation. However, although not evaluated in the present study, waiting until mid-summer to start feeding a very high NDFd forage can become challenging due to aerobic instability, rapid spoilage, and additional DM losses. If ensiled Triticale feeding is targeted for that time, it is clear that the greater acetic production of the heterofermentative inoculant (SiloSolve® FC) may be the logical choice for ensiling. Future research with Triticale should investigate more broadly the impact of homo- vs. heterofermentative inoculants across a variety of DM levels and ensiling conditions.

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***Lacticaseibacillus parahuelsenbergensis* and *Lacticaseibacillus styriensis*, Isolated from Grass and Corn Silage**

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Keywords: *Lacticaseibacillus parahuelsenbergensis*, *Lacticaseibacillus styriensis*, lactic acid bacteria, new species, silage

Introduction

In a screening project, two rod-shaped, non-motile, non-spore-forming, facultative anaerobic, Gram-stain positive lactic acid bacteria, designated as LD0937^T and SCR0063^T, were isolated from perennial grass (1st cut) and corn silage. Polyphasic approach characterisation identified these as novel species of the *Lacticaseibacillus* genus [1].

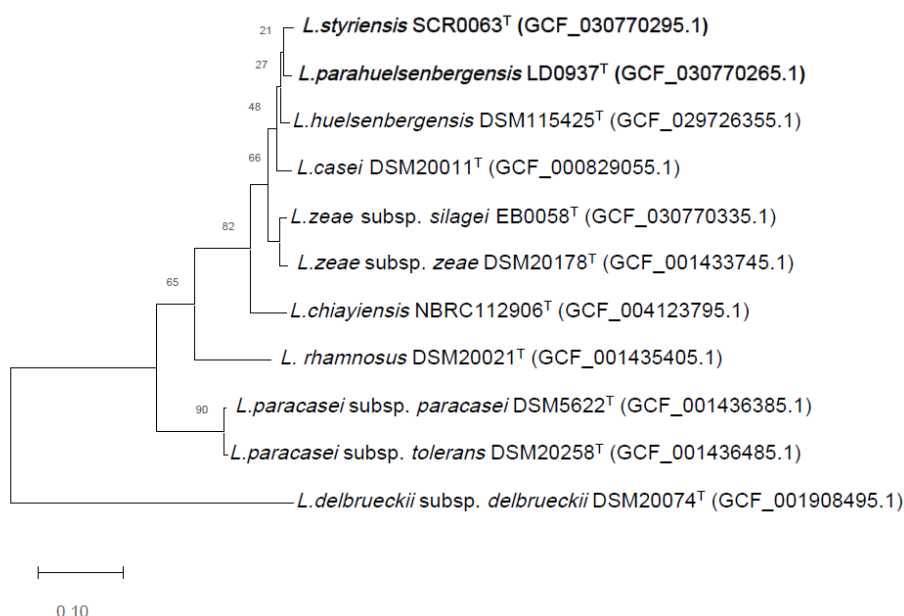
Materials and methods

A core gene phylogenetic tree was constructed using the UBCG ver3.0 tool [2] and default options (Figure 1). The tree was visualized using the MEGA 11 software (version 11.0.3.) [3].

For phenotypic characterisation, the novel strains and all reference bacteria were cultivated on MRS agar plates at 37°C for 24 h under semi-anaerobic conditions in an anaerobic box with anaerogen bags. Growth tests were conducted in MRS broth with a range of parameters, including pH (3.0 to 10.5, interval 0.5) and NaCl salt concentrations (4% w/v to 10% w/v, interval 1% w/v). Using the commercially available test kit API 50 CH system (bioMérieux), the capacity for carbohydrate metabolism was measured (Table 1).

Results

Figure 1. Phylogenetic analysis of the novel isolates LD0937^T and SCR0063^T core genome using UBCG tool. Reference strains are the phylogenetically closest related *Lacticaseibacillus* type strains. *Lactobacillus delbrueckii* subsp. *delbrueckii* DSM 20074^T served as outgroup.



We will focus the results presentation on variables significantly different among strains. According to the analysis of the core genome, the isolates LD0937^T and SCR0063^T form two distinct clusters within the *L. casei* group (Figure 1). Thus, the isolates can be identified as novel species, *L. parahuelsenbergensis* LD0937^T and *L. styriensis* SCR0063^T.

Table 1. Differential phenotypic and growth characteristics of the two isolates and their phylogenetically closest related type strains *L. huelsenbergensis* DSM 115425^T and *L. casei* DSM20011^T.

Strain	Growth at:			Acid production from:			
	pH 3.5	pH 10.0	8% NaCl	L-Arabinose	α-methyl-D-Mannoside	L-Sorbose	Dulcitol
LD0937 ^T	w	+	+	+	-	+	-
SCR0063 ^T	+	+	w	+	+	-	-
<i>L. huelsenbergensis</i> DSM115425 ^T	+	-	w	-	-	-	+
<i>L. casei</i> DSM20011 ^T	-	-	-	-	-	-	-

+ ... good growth, w ... weak growth, - ... no growth

Growth and distinctive features among the strains LD0937^T, SCR0063^T, their closest relatives *L. huelsenbergensis* DSM 115425^T and *L. casei* DSM20011^T can be found in Table 1. Strain LD0937^T showed weak growth at pH 3.5 and good growth at pH 10.0 and in medium containing 8% w/v NaCl, whereas SCR0063^T showed weak growth in medium containing 8% w/v NaCl and good growth at pH 3.5 to pH 10.0. The results from the novel strains contrast significantly with the closest related strains *L. huelsenbergensis* DSM 115425^T and *L. casei* DSM 20011^T. LD0937^T and SCR0063^T were able to produce acid from L-Arabinose, LD0937^T from L-Sorbose and SCR0063^T from α-methyl-D-Mannoside, which differed from the characteristics of their nearest phylogenetic relatives. Furthermore, only *L. huelsenbergensis* DSM 115425^T was able to metabolize Dulcitol in contrast to the two novel isolates and the type strain of *L. casei*.

Conclusions

Based on this polyphasic approach, using genomic, phylogenetic, and phenotypic analysis, LD0937^T and SCR0063^T represent two novel species within the *Lacticaseibacillus* group: *Lacticaseibacillus parahuelenbergensis* with the type strain LD0937^T (=DSM 116105 =NCIMB 15471) and *Lacticaseibacillus styriensis* with the type strain SCR0063^T (=DSM 116297 =NCIMB 15473). The role of these novel species as a potential silage additive is further investigated.

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Lacticaseibacillus zeae Subsp. silagei, Isolated from Corn Silages

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Keywords: *Lacticaseibacillus zeae*, lactic acid bacteria, new subspecies, corn silage.

Introduction

In a screening project, two rod-shaped, non-motile, non-spore-forming, facultative anaerobic, Gram-stain positive lactic acid bacteria, designated as EB0058^T and SCR0080, were isolated from untreated corn silages. They were characterised using a polyphasic approach and identified as novel subspecies of *Lacticaseibacillus zeae* [1].

Materials and methods

In order to detect novel species, Chun, Oren et al. proposed the computation of the overall genome-related index (OGRI) [2]. The Genome-to-Genome Distance Calculator calculated the dDDH value with recommended formula 2 [3,4]. The ANI values were calculated using the JSpecies Web Server with BLAST+ mode [5] (Figure 1). For phenotypic characterisation, the novel strains and all reference bacteria were cultivated on MRS (Man Rogosa Sharpe) agar plates at 37°C for 24 h under semi-anaerobic conditions in an anaerobic box with anaerogen bags. Growth tests were conducted in MRS broth with a range of parameters, including temperature (5 to 55°C, interval 5°C), pH (3.0 to 10.5, interval 0.5), NaCl salt concentrations (4% w/v to 10% w/v, interval 1% w/v) and KCl salt concentrations (6% w/v, 8.4% w/v and 10.8% w/v). Using the commercially available test kit API 50 CH system (bioMérieux), the capacity for carbohydrate metabolism was measured (Table 1).

Results

We will focus the results presentation on variables significantly different among strains.

Figure 1. ANI and dDDH (formula 2) values for strains EB0058^T, SCR0080 and related species.

	Group A				Group B								
	<i>L. zeae</i> subsp. <i>silagei</i>				<i>L. zeae</i> subsp. <i>zeae</i>								
	1	2	3	4	5	6	7	8	10	11	12	13	
[1] <i>L. zeae</i> subsp. <i>silagei</i> EB0058 ^T	-	99.9	99.9	99.9	96.3	96.3	96.3	96.4	93.7	93.6	93.7	94.1	ANI ↑
[2] <i>L. zeae</i> subsp. <i>silagei</i> SCR0080	99.6	-	99.9	99.9	96.3	96.3	96.2	96.3	93.7	93.6	93.8	93.8	
[3] <i>L. zeae</i> subsp. <i>silagei</i> UD2202	99.8	99.9	-	99.9	96.3	96.2	96.2	96.3	93.7	93.6	93.7	93.7	
[4] <i>L. zeae</i> subsp. <i>silagei</i> CECT9104	99.8	99.9	99.3	-	96.3	96.3	96.2	96.3	93.7	93.6	93.8	93.7	
[5] <i>L. zeae</i> subsp. <i>zeae</i> DSM 20178 ^T	70.8	70.7	70.6	70.7	-	100	99.3	99.5	93.6	93.5	93.5	94.0	
[6] <i>L. zeae</i> subsp. <i>zeae</i> KCTC 3804 ^T	70.8	70.7	70.5	70.7	100	-	99.2	99.5	93.6	93.5	93.5	93.9	
[7] <i>L. zeae</i> subsp. <i>zeae</i> CRBIP24.58	70.5	70.2	70.3	70.4	95.6	95.5	-	99.5	93.6	93.5	93.5	93.7	
[8] <i>L. zeae</i> subsp. <i>zeae</i> FBL8	71.5	71.5	71.3	71.5	95.9	95.7	97.3	-	93.6	93.5	93.6	93.9	
[10] <i>L. parahuelsenbergensis</i> LD0937 ^T	54.2	54.1	53.8	54.2	53.9	54.1	54.3	54.4	-	95.9	95.7	95.2	
[11] <i>L. styriensis</i> SCR0063 ^T	54.8	54.7	54.5	54.8	54.6	54.8	54.4	55.0	67.6	-	95.9	95.2	
[12] <i>L. huelsenbergensis</i> DSM 115425 ^T	54.3	54.2	53.9	54.2	53.4	53.5	53.4	54.2	66.5	65.9	-	95.4	
[13] <i>L. casei</i> DSM 20011 ^T	57.1	56.9	56.5	56.9	57.3	57.4	56.0	57.4	64.1	64.9	63.9	-	
	dDDH <-												

The cut-off values for a novel subspecies are an average nucleotide identity (ANI) value between 96-97% or a digital DNA-DNA-hybridisation (dDDH) value between 70-80%. Due to whole-genome sequence-based characterization, these two strains were separated into a distinct clade from *Lacticaseibacillus zeae* DSM 20178^T, together with CECT9104 and UD2202, whose genomic sequences are available at NCBI GenBank. The ANI values within the new subgroup A are 99.9% and the dDDH values are 99.3% - 99.9%, respectively. In contrast, comparing the new subgroup to all publicly available genomic sequences of *L. zeae* strains including the type strain DSM 20178^T showed dDDH values between 70.2% - 72.5% and ANI values between 96.2% - 96.3% (Figure 1).

Table 1. Differential phenotypic and growth characteristics of the two isolates and their phylogenetically closest related type strain *L. zeae* DSM 20178^T.

Strain	Growth at:				Acid production from:			
	5°C	pH 10	9% NaCl	8.4% KCl	L-Arabinose	Sorbitol	Maltose	D-Arabitol
EB0058 ^T	+	-	+	+	+	+	+	+
SCR0080	+	-	+	+	+	+	+	+
<i>L. zeae</i> DSM20178 ^T	-	+	-	w	-	-	-	-

+ ... good growth, w ... weak growth, - ... no growth

Growth and distinctive features among the strains EB0058^T, SCR0080 and the closest phylogenetically related type strain *L. zeae* DSM 20178^T can be found in Table 1. The two isolates show totally different growth behavior than the type strain *L. zeae* DSM 20178^T. Both isolates can grow at 5°C and with 9%w/v NaCl and 8.4% w/v KCl, but they cannot grow at pH 10, in contrast to their nearest phylogenetic relative. EB0058^T and SCR0080 could produce acid from L-arabinose, sorbitol, maltose and D-arabitol which differed from the characteristics of the nearest phylogenetic neighbor *L. zeae* DSM 20178^T.

Conclusions

Based on their chemotaxonomic, phenotypic, and phylogenetic characteristics, EB0058^T and SCR0080 represent a new subspecies of *L. zeae*. The name *Lacticaseibacillus zeae* subsp. *silagei* is proposed with the type strain EB0058^T (=DSM 116376 =NCIMB 15474). The role of this novel subspecies as a potential silage additive is further investigated.

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Citrus Essential Oil as Silage or Feed Additive Has No Effect on Sheep Feed Intake and Daily Gain

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Keywords: animal performance, orange, ruminants, tropical silage

Introduction

Sugarcane can be used as roughage in the feeding of ruminants. When used in the form of silage, it promotes its use by concentrating operational activities and also facilitates the proper management of the cane field (Campana et al., 2021). However, sugarcane silage presents fermentative problems that can be minimized with the use of additives. Data from the literature demonstrate that essential oils (EO) can beneficially aid this process. Limonene is a citrus EO that has great potential for use as an additive in ruminant feeding. It can bring benefits to the fermentation of sugarcane silage and, when added directly to the diet, has positive effects on animal health and performance.

The use of EOs individually (without being in mixtures) can also present benefits that can positively affect digestibility, ruminal fermentation, and milk composition for ruminants (EL-ESSAWY et al. 2021). There was an improvement in the digestive ability of the rumen through impacts on its microorganisms when OE as anise, cloves, thyme, oregano, and others, when supplied individually to cattle cut (ZHANG et al., 2021). Júnior et al. (2024) point out that citrus EO can be used to feed ruminants increasing live weight gain and benefiting animal carcasses, also reducing methane emissions.

Therefore, the objective of this work is to evaluate the performance of sheep fed with sugarcane silage, which is enhanced during ensiling and/or feeding with citrus EO.

Materials and methods

Eight Dorper lambs were used in a trial using a 4 × 4 Latin Square design to evaluate four treatments. Treatments were obtained from a 2 × 2 factorial arrangement designed to evaluate the following factors: I. Citrus essential oil addition during the sugarcane ensiling: 0 (SS) and 750 mg citrus essential oil / kg of DM sugarcane (SC); and II. Citrus essential oil addition during the animals feeding: 0 (S) and 750 mg (C) per kg of diet DM. Diets were formulated to 150 g / day of average daily weight gain according to Small Ruminant Nutrition System (SRNS) model and animals were fed once daily to allow 10% of daily orts. Each experimental period had 18 days, with 13 days of adaptation to treatments and five days of data and sample collection. The weight of the animals was measured at the beginning and end of each experimental period. Protocols are followed by Del Valle et al., (2024).

Results

Table 1. Dry matter intake and daily gain of sheep feed diets containing citrus essential oils added during sugarcane ensiling and during the feeding.

Item	Treatments				SEM ⁵	Probabilities ⁶		
	SCC ¹	SCS ²	SSC ³	SSS ⁴		SIL	F	SIL×F
Dry matter intake								
g/day	1.08	1.11	1.09	1.06	0.021	0.528	0.926	0.311
% BW	3.58	3.70	3.62	3.47	0.128	0.349	0.889	0.192
ADG, g	155	174	171	175	8.3	0.589	0.472	0.643
Body weight, kg	30.5	30.5	30.5	30.7	0.93	0.320	0.585	0.535

¹SCC: sugarcane silage with citrus essential oil and animals receiving CEO on diet;

²SCS: sugarcane silage with CEO and animals without CEO during the feeding;

³SSC: sugarcane silage without CEO and animals receiving CEO on diet;

⁴SSS: sugarcane silage without CEO and animals without CEO during the feeding;

⁵Standard error of mean;

⁶Probabilities: SIL: effect of CEO addition during the ensiling; F: effect of CEO addition during the animals feeding; and SIL × F: interaction of ensiling and feeding effect of CEO supply.

Conclusions

The use of Citrus EO addition in sugarcane silage or feed additive does not affect sheep feed intake and daily gain.

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Fermentative Losses of Sugarcane Silage Treated with Increasing Levels of Citrus Essential Oil

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Keywords: dry matter recovery, gas losses, limonene, tropical silages

Introduction

Sugarcane has a wide distribution in Brazil and is one of the country's main crops. In addition to being used for sugar and ethanol production, it is also employed as cattle feed, especially during the dry season, when it can be provided fresh or as silage. However, sugarcane harvesting requires intensive labor for daily cutting, which negatively affects crop management and longevity (Campana et al., 2021). One solution to minimize the challenges of daily harvesting is whole-plant ensiling. This process allows for a one-time harvest, reducing losses caused by fires or frosts and extending the useful life of sugarcane fields (Pedroso et al., 2007).

However, sugarcane presents challenges that hinder the production of high-quality silage. In this context, additives and inoculants can be used to improve silage fermentation and nutritional value. Various additives, including chemicals and essential oils (EO), have antifungal effects and contribute to sugarcane silage preservation (Cantoia Jr. et al., 2020). Among the traditional EOs used in animal feeding and silage preservation, sweet orange essential oil (*Citrus sinensis* (L.) Osbeck) is widely available. This EO contains over 300 different chemical compounds, with the most abundant being limonene (94%). Limonene is primarily responsible for the oil's antifungal properties (Marriott et al., 2001), making it a promising candidate as a silage inoculant. The objective of this study was to evaluate the fermentative losses of sugarcane silage added with citrus essential oil.

Materials and methods

Thirty-two experimental silos were used in a blocked-randomized design to evaluate the following treatments: 0, 250, 500 and 750 mg of citrus essential oil/ kg DM of fresh sugarcane. Storage was performed for 100 days. Silos were weighted several times during the storage time.

Fermentation losses were calculated according to JOBIM et al. (2007) considering: silo weight without silage at opening; silo weight without silage at ensiling, and ensiled dry matter, whole silo weight (within silage) at ensiling and whole silo weight (within silage) at silos opening. Total fermentation losses (TFL, g/kg) was calculated as effluent production and gas losses sum. Dry matter recovery (DMR, g/kg) was obtained by opening DM (g) to ensiled DM (kg) ratio.

Results

Table 1. Losses, and dry matter recovery of sugarcane silage treated with increasing levels of citrus essential oil.

Item	Treatments ¹				SEM ²	Probabilites ³			
	0	250	500	750		Treat.	Lin.	Qua.	Des.
Effluent losses									
g/kg DM	266	296	285	251	20.5	0.418	0.545	0.132	0.844
Gas losses									
g/kg DM	145	133	142	133	7.1	0.830	0.609	0.900	0.448
Total losses									
g/kg DM	394	429	427	384	23.1	0.301	0.728	0.068	0.953
Dry matter recovery									
g/kg DM	720	704	698	730	8.6	0.520	0.753	0.161	0.717

¹Increasing levels of citrus essential oil (0, 250, 500, and 750 mg/kg DM); ²Standard error of mean; ³Probabilities: Treat.:

Treatments effect: linear (Lin.), quadratic (Qua.), quadratic deviation (Des.) effect of citrus EO.

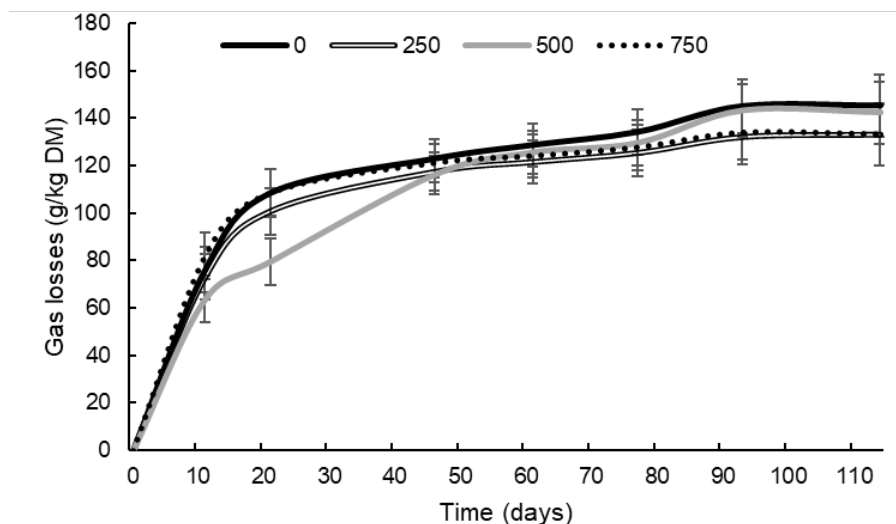


Figure 1. Effects on increasing levels of citrus essential oil on sugarcane silage gas losses after ensiling.

Conclusions

The inclusion of citrus essential oil did not affect fermentative losses of sugarcane.

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Preharvest Management Considerations to Optimize Silage Quality

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Keywords: whole-plant corn silage, digestibility, fungus

Introduction

On a DM basis, whole-plant corn silage (WPCS) composition is about 60% corn ears, 12% corn leaves, and 28% corn stalks (Kuehn et al., 1999). For dairy cattle diets, WPCS represents 40 to 60% of the total mix ration in lactating diets (Mueller and Wise, 2014). One of the many parasites that can affect corn plants is fungi. The triangle of disease is composed of the host (in this case, the corn plant), the pathogen (fungi), and the environment (i.e., weather, soil). Corn plant physical barriers, such as cell walls and chemical releases (i.e., secondary metabolites), aid plants in protecting themselves from pathogens (Malinovsky et al., 2014). Fungi maintain tissue growth by obtaining nutrients from the plant. That is accomplished consistently if fungi remain undetected on the plant surface, where enzymes degrade cell walls, and once inside, produce toxins killing the plant tissue (Sexton and Howlett, 2006). Plants have adapted by increasing the lignin concentration in the secondary cell wall, thus creating a thicker layer for digesting when distressed or infected with a fungal pathogen or insect (Santiago et al., 2013). However, once inside the cell, and growth has ceased, fungi release secondary metabolites, which in some species are toxic. It is generally hypothesized that during the colonization and sporulation phase of a fungus within a plant, mycotoxins are secreted by growing colonies (Calvo et al., 2002). Therefore, the objectives of the present work were to summarize the existing knowledge on the relationship between fungi and plants, to limit plant infection by applying fungicides, and to investigate how WPCS from corn plants with fungicide application affects dairy cow performance.

Results

Under pressure from fungal disease, application of pyraclostrobin (Headline, BASF Corp., Florham Park, NJ) on corn plants at the vegetative stage increased grain yield by 550 kg/ha compared with untreated fields of corn plants (Nelson and Meinhardt, 2011). But others (Mueller and Pope, 2009; Wright et al., 2014) have reported earlier applications to be beneficial as well. In a year with high incidence of common rust, foliar fungicide applied as a preventative at V6 increased corn grain yield by 362.9 kg/ha compared with application at before tassel, when 6% of the total leaf area was diseased (Wright et al., 2014). Yet, in a different year of the same study, when disease incidence was low, foliar fungicide applied as a preventative at V6 did not increase corn grain yield when compared with application at tassel (Wright et al., 2014). Fungicide applications on corn plants can improve the nutrients within the plant material. A study at the University of Illinois evaluated the effects of fungicide application on the physical and nutritional content of corn plant leaves, ears, stalks, and flag leaves (Kalebich et al., 2017). Fungicide applications on corn plants during the summer of 2015 were as follows: control (CON), corn receiving no foliar fungicide application; treatment 1 (V5), where corn plants received a mixture of pyraclostrobin and fluxapyroxad (PYR+FLUX) foliar fungicide at V5; treatment 2 (V5+R1), where corn plants received 2 applications of foliar fungicide, a mixture of PYR+FLUX at V5 and a mixture of pyraclostrobin + metconazole (PYR+MET) foliar fungicide at corn reproductive stage 1 (R1); treatment 3 (R1), in which corn plants received one application of PYR+MET foliar fungicide at R1. Corn plants with fungicide treatment were taller compared with untreated (2.7, 2.9, 3.0, and 2.9 m for CON, V5, V5+R1, and R1). Corn leaves in V5+R1 and in R1 had less yellow lower leaves than in CON and V5 (0.85, 0.77, 0.42, and 0.44 leaves for CON, V5, V5+R1, and R1, respectively). Corn stalks in V5+R1 had greater lignin concentration compared with CON and R1 (4.6, 5.6, 6.4, and 5.0% of DM for CON, V5, V5+R1, and R1). Corn leaves in V5+R1 had lower ADF and NDF concentrations (28.3 and 52.4% for ADF and NDF, respectively) compared with leaves in CON (33.3 and 56.9% for ADF and NDF, respectively; Kalebich et al., 2017). Additionally, the group determined the effects of foliar fungicide (FUN; Headline AMP; BASF Corp., applied at vegetative tassel growth stage) and ensiling time (0, 30, 90, and 150 d) on fiber composition of 2 corn varieties [brown midrib (BMR) and flourey (FLY); Hollis et al., 2019]. Treatments were assigned to sixteen 3.38-ha plots in a completely randomized split-plot block design. Treatments were BMR without FUN, FLY without FUN, BMR with FUN, and FLY with FUN. Samples of whole corn plants were collected and separated into leaves, stalks, flag leaf, and cobs. Corn plants in CON exhibited greater incidence of GLS disease than FUN corn plants with 6.52 and 1.75 ± 0.16% of leaf area, respectively. Corn plants in CON exhibited greater incidence of ear leaf injury than FUN with 2.74 and 0.72 ± 0.13% of leaf area, respectively. Corn plants in CON exhibited greater incidence of ear leaf injury at one leaf below the highest ear leaf compared with FUN with at 3.97 and 1.22 ± 0.17%, respectively. Brown midrib corn plants had a greater number of green leaves than FLY with 11.81 and 11.34 ± 0.09 leaves, respectively. Corn plants in control (BMR without FUN and FLY without FUN) had a greater number of yellow leaves than FUN corn plants with 0.28 and 0.08 ± 0.02, respectively. Most recently, our group determined the effects of cut height at harvest and foliar fungicide application on BMR WPCS yield, chemical composition, and in situ degradability (Damery, 2018). Foliar fungicide (prothioconazole and trifloxystrobin; Delaro, Bayer Crop Science, Monheim am Rhein, Germany) treatments were randomly assigned to one of sixteen 0.21-ha plots as follows: control (CON), plants received no application; treatment 1 (V5), plants received one application at corn vegetative stage 5 (V5); treatment 2 (V5R1), plants received 2 applications at V5 and corn reproductive stage 1 (R1); treatment 3 (R1), plants received one application at R1. At R5, corn plants in R1 and V5R1 had less yellow leaves (0.35 and 0.47 ± 0.19, respectively) than CON and V5 (0.63 and 1.08 ± 0.19, respectively). Disease prevalence was recorded as percentage of the total individual plant infected. Fungicide application had no effect on disease prevalence (1.62, 1.07, 1.23, 1.48 ± 0.30% for CON, V5, V5R1, and R1, respectively). There was no effect of fungicide treatment or cut height on the degradability of OM, NDF, or ADF. Raising the cut height from low cut (30.5

cm) to high cut (55.9 cm) increased the effective degradability of DM (0.565 and 0.577 ± 0.01 , respectively), CP (0.565 and 0.576 ± 0.01 , respectively), and starch (0.825 and 0.847 ± 0.01 , respectively).

Conclusions

It is well known that the responsible application of foliar fungicides on corn plants helps limit devastating yield losses. Cows fed WPCS from corn plants with fungicide application improved feed efficiency and were more profitable than cows fed WPCS with no fungicide application. Whole-plant corn silage from corn plants with fungicide application reduced the fiber concentration, thereby improving its nutritive value. Overall, fungicide application seems to be maximized when converted into feed (e.g., WPCS) and fed to dairy cattle.

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Effect of Replacing Hexamine by Sodium Formate in Nitrite-Based Additives for Palisade Grass Silage

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Keywords: chemical additive, clostridia, fermentation, tropical grass

Introduction

Silage additives based on sodium nitrite combined with hexamine have proven effective in improving fermentation in tropical grass silage (Gomes et al., 2021; Moraes et al., 2023). However, due to cost and regulatory issues related to hexamine there has been an increased interest in replacing this component with alternative substances. A recent meta-analysis suggested that sodium formate may effectively replace hexamine in temperate silages without compromising fermentation quality (Auerbach et al., 2023). However, its effectiveness has not been tested in tropical grass silages. This study aimed to examine if sodium formate can replace hexamine in an additive based on sodium nitrite to improve fermentation and reduce dry matter (DM) loss in palisade grass silage.

Materials and methods

Palisade grass (*Urochloa brizantha* cv. Marandu) was cut after 56 d of regrowth (March 2022) at 10 cm above soil level, and chopped by a stationary forage chopper (10 mm of theoretical length of cut) (DM = 25.3% FM; ethanol-soluble carbohydrates = 3.60% DM; buffering capacity = 3.19 g lactic acid 100 g DM⁻¹; fermentability coefficient = 34.3). The chopped forage was divided into 20 piles (5 kg per pile) to prepare small-scale silos (7-L plastic buckets). The treatments were as follows: 1) control (without additives); 2) sodium nitrite (0.6 g kg FM⁻¹) combined with hexamine (0.4 g kg FM⁻¹) (NHM); 3) sodium nitrite (0.9 g kg FM⁻¹) combined with hexamine (0.6 g kg FM⁻¹) (NHH); 4) sodium nitrite (0.6 g kg FM⁻¹) combined with sodium formate (0.4 g kg FM⁻¹) (NFM); and 5) sodium nitrite (0.9 g/kg) combined with sodium formate (0.6 g/kg) (NFH). After 90 d of storage at room temperature (14 to 32 °C), silages were sampled to determine fermentation profile by standard wet chemistry and microbial counts by routine microbiological procedures. Ammonia-N concentration (% of total N) was corrected for the addition of N by the additive. The loss of DM was calculated according to Weissbach (2005). Data were analyzed as a completely randomized design using the Mixed procedure of SAS. Means were compared by orthogonal contrasts: CON vs. ADT = Control vs. (NFM + NFH + NHM + NHH), NH vs. NF = (NFM + NFH) vs. (NHM + NHH). Significance was declared at $P \leq 0.05$.

Results

Additives were effective to inhibit *Clostridium* development and improved the fermentative quality of palisade grass (Table 1). Treated silages had greater concentrations of lactic acid and lower concentrations of acetic, propionic and n-butyric acids compared with the control. Consequently, DM loss during fermentation was markedly reduced by additives, thereby improving the efficiency of the fermentation process. This suggests that the addition of sodium nitrite in combination with either hexamine or sodium formate contributed to minimizing the anaerobic degradation of those silages. The NH₃-N concentration was significantly lower in treated silages compared with the control, particularly in the NHH and NFH treatments (i.e., the higher additive doses). This indicates a lower degree of proteolysis during the fermentation process, attributed to the inhibition of the activity of clostridia and other proteolytic bacteria. The almost complete absence of n-butyric acid in all additive treatments supports this finding, since this compound is a typical marker of clostridia activity (Weissbach et al., 1974). Furthermore, *Clostridium* counts were reduced by the additives, with the sodium formate being more effective than hexamine.

Conclusions

Sodium formate was effective in replacing hexamine in a sodium nitrite-based additive for palisade grass silage.

Table 1. Microbial counts, fermentation profile and dry matter loss of palisade grass silages stored for 90 d

	Treatment ¹					SEM ²	P-contrast ³			
	CON	NFM	NFH	NHM	NHH		CON vs. ADT	NH vs. NF	NHM vs. NHH	NFM vs. NFH
Dry matter, %FM	22.4	25.1	25.5	25.2	25.3	0.17	<0.01	0.77	0.68	0.11
Clostridia, log cfu g FM ⁻¹	3.37	2.00	1.82	2.08	2.23	0.109	<0.01	0.04	0.37	0.26
pH	4.62	4.57	4.40	4.43	4.74	0.084	0.36	0.26	0.02	0.17
NH ₃ -N, %N	29.4	16.3	13.9	16.5	11.9	0.74	<0.01	0.23	<0.01	0.03
Lactic acid, %DM	0.573	2.55	3.33	3.41	3.67	0.160	<0.01	<0.01	0.28	<0.01
n-Butyric acid, %DM	2.30	0.020	0.023	0.005	<0.001	0.063	<0.01	0.77	0.96	0.98
Acetic acid, %DM	2.19	2.43	1.64	0.893	0.629	0.152	<0.01	<0.01	0.23	<0.01
Propionic acid, %DM	0.525	0.176	0.074	0.011	0.009	0.013	<0.01	<0.01	0.79	<0.01
DM loss, %DM	9.17	2.74	2.22	2.60	2.12	0.046	<0.01	0.02	<0.01	<0.01

¹CON: Control (without additive), NHM: Sodium nitrite (0.6 g kg FM⁻¹) + Hexamine (0.4 g kg FM⁻¹), NHH: Sodium nitrite (0.9 g kg FM⁻¹) + Hexamine (0.6 g kg FM⁻¹), NFM: Sodium nitrite (0.6 g kg FM⁻¹) + Sodium formate (0.4 g kg FM⁻¹), NFA: Sodium nitrite (0.9 g kg FM⁻¹) + Sodium formate (0.6 g kg FM⁻¹).

²Standard error of the mean.

³Contrast: CON vs. ADT = Control vs. (NFM + NFH + NHM + NHH), NH vs. NF = (NFM + NFH) vs. (NHM + NHH).

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Effects of Dry Matter Content and Heterofermentative Inoculant on the Conservation of Whole-Plant Corn Silage

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Keywords: aerobic stability, fermentation, lactic acid bacteria, yeast

Introduction

Whole-plant corn silage is the main ensiled forage worldwide. In the last growing seasons, corn crops experienced agronomical challenges in different regions of South America, such as greater risk of drought and increased occurrence of pests (e.g., leafhopper) and diseases. Consequently, corn plants are being harvested and ensiled with a higher dry matter (DM) content (>40% DM), which may increase the risk of aerobic deterioration. Meanwhile, some farmers are applying water to partially rehydrate chopped corn forage before ensiling, sometimes in combination with the use of heterofermentative inoculants to improve aerobic stability. Therefore, the objective of this study was to evaluate the effects of an inoculant based on heterolactic bacteria on the fermentation, chemical composition, aerobic stability and *in situ* ruminal degradability of whole-plant corn silage that underwent water stress and leafhopper attack, ensiled with different DM contents.

Materials and methods

Whole-plant corn was harvested at two maturity stages. The first harvest was performed when the corn plants had approximately 32% of DM (**DM32**) and the second harvest was performed when the corn plants reached approximately 42% of DM. During the second harvest, one-half of the forage was ensiled with its original DM (**DM42**) whereas the other forage half was rehydrated in order to reach 32% of DM (**DM32R**). Forage from all three DM conditions were ensiled after application of 1.5×10^5 CFU g⁻¹ of *Lentilactobacillus buchneri* NCIMB 40788 + 1.5×10^5 CFU g⁻¹ of *Lentilactobacillus hilgardii* CNCM I-4785 (Magniva Platinum 1, Lallemand; **LBLH**) or nothing (**Control**), with four replicates per treatment. The inoculant was diluted in distilled water and applied at 10 mL kg⁻¹. The control received the same volume of water. After manual homogenization, 5.2 kg of treated forage was ensiled in 7.2 L plastic buckets (experimental silos). The factorial combination of three DM levels, two additives (control and inoculant) and four replicates resulted in 24 experimental silos. After 90 d of storage, silages were sampled to evaluate the DM loss, fermentative profile, *in situ* ruminal degradability (unprocessed samples), chemical composition, and aerobic stability. Data were analyzed using the MIXED procedure of SAS as a completely randomized design with a 3 × 2 factorial arrangement. Means were compared by Tukey-Kramer test ($\alpha = 0.05$).

Results

Inoculation with LBLH resulted in higher counts of LAB among silages with different DM content, and lower counts of yeast for DM32 and DM42 compared to the control. Mold counts were relatively higher for DM42 and DM32R silages compared with DM32. Treating DM32R silage with LBLH resulted in lower concentration of lactic acid and higher concentration of acetic acid. Formation of 1,2-propanediol induced by LBLH was greater in DM32 and DM32R. Silage pH was lower in DM32 and higher in DM42, with intermediate values for DM32R, but DM loss during fermentation was not affected by treatment. As consequence of the fermentation pattern, DM32 and DM32R silages inoculated with LBLH showed higher aerobic stability. Ruminal degradability of DM at 12 h and 24 h incubations were lower for DM42 compared to DM32, with intermediate values for the rehydrated silage (DM32R). As expected, ruminal degradability of NDF at 24 h was lower for DM42 and DM32R compared to DM32. Late harvest (DM42) also decreased ruminal starch degradability compared with early harvest (DM32). However, silage rehydration (DM32R) and inoculation with LBLH improved ruminal starch degradability, likely due to the greater bacterial activity and then prolamin degradation in kernel endosperm (Junges et al., 2017). For instance, corn silage that was rehydrated (DM32R) and inoculated with LBLH had the same ruminal starch degradation than the early harvested (DM32) untreated silage.

Table 1. Fermentation pattern, DM loss, aerobic stability, and *in situ* ruminal degradability of corn silages

Item	Treatment ¹							<i>P</i> -value ³		
	Control			LBLH						
	DM32	DM32R	DM42	DM32	DM32R	DM42	SEM ²	M	I	M×I
Dry matter, % FM	29.9 ^c	32.1 ^b	40.8 ^a	29.1 ^c	32.8 ^b	40.7 ^a	0.34	<0.01	0.89	0.29
LAB, log CFU g-1 FM	6.11	6.71	7.20	8.17	7.99	7.82	0.353	0.60	<0.01	0.18
Yeasts, log CFU g-1 FM	4.15 ^a	3.14 ^{cd}	4.03 ^{ab}	3.32 ^{bc}	2.44 ^d	3.08 ^{cd}	0.169	<0.01	<0.01	0.79
Molds, log CFU g-1 FM	2.74 ^{cd}	3.40 ^{abc}	4.11 ^a	2.16 ^d	3.13 ^{bc}	3.78 ^{ab}	0.146	<0.01	<0.01	0.59
pH	3.61 ^c	3.71 ^b	3.79 ^a	3.59 ^c	3.70 ^b	3.80 ^a	0.008	<0.01	0.306	0.46
Lactic acid, % DM	7.85 ^a	6.46 ^b	4.13 ^d	7.29 ^{ab}	5.32 ^c	4.18 ^d	0.217	<0.01	<0.01	0.04
Acetic acid, % DM	2.96 ^b	1.84 ^d	1.67 ^d	3.46 ^a	2.39 ^c	1.89 ^d	0.085	<0.01	<0.01	0.17
1,2-Propanediol, mg kg ⁻¹ DM	90.1 ^b	46.5 ^b	21.6 ^b	3662 ^a	4763 ^a	584 ^b	484.0	<0.01	<0.01	<0.01
DM loss, %	4.28	4.12	4.14	4.42	4.03	4.16	0.126	0.11	0.80	0.65
Aerobic stability, d	63.0 ^b	44.8 ^b	51.4 ^b	152 ^a	126 ^a	80.4 ^b	11.55	<0.01	<0.01	0.04
In situ starch degradability 12h, %	87.4 ^b	85.8 ^c	82.6 ^d	89.9 ^a	87.2 ^b	82.4 ^d	0.62	<0.01	0.03	0.10
In situ DM degradability 12h, %	57.7 ^a	54.7 ^{ab}	50.1 ^b	58.3 ^a	55.7 ^{ab}	50.2 ^b	1.98	<0.01	0.73	0.97
In situ NDF degradability 24h, %	49.1 ^a	38.5 ^b	35.1 ^b	48.9 ^a	39.4 ^b	35.3 ^b	2.79	<0.01	0.89	0.98
In situ DM degradability 24h, %	65.2 ^a	61.9 ^{ab}	58.7 ^b	65.7 ^a	63.1 ^{ab}	58.7 ^b	1.18	<0.01	0.71	0.95

¹Control: without addition of inoculant; LBLH: inoculation with 1.5×10^5 CFU g⁻¹ of *Lentilactobacillus buchneri* + 1.5×10^5 CFU g⁻¹ of *Lentilactobacillus hilgardii*. ²Standard error of the mean. ³M: dry matter content; I: inoculant; M×I: interaction between dry matter and inoculant.

Conclusions

Inoculation with 1.5×10^5 CFU g⁻¹ of *Lentilactobacillus buchneri* + 1.5×10^5 CFU g⁻¹ of *Lentilactobacillus hilgardii* resulted in a substantial increase in the aerobic stability of corn silage harvested with regular DM content (32% DM) or harvested with high DM content and rehydrated to reach regular DM content (32% DM). Silage rehydration and inoculation with heterofermentative inoculant also improved ruminal starch degradability. Then, the combination of heterolactic inoculant and rehydration may be a strategy to improve the quality of corn silage harvested with high DM content. When rehydration is considered for late harvested corn plants, farmers should be aware of the water volume required and evenly distribute such water into forage mass to avoid percolation across silage layers.

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Survey of Fiber Quality of Cover Crops Used as Forage to Dairy Cattle in Wisconsin

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Keywords: forage, in vitro, sustainability.

Introduction

Small grain forages continue to rise in popularity in many parts of the United States. These crops have garnered interest for multiple reasons. Many farmers plant these crops primarily as cover crops to improve soil health and nutrient management while reducing erosion and nutrient losses. Planting small grain cover crops between fall harvest and spring planting adds versatility and allows dairies to produce extra forage, which can allow them to stretch forage inventories. Although a small grain harvest requires additional labor, equipment, and seed, the extra silage can restore forage inventories when less than ideal weather patterns decrease the yield of primary forage crops. The objective of this study was to assess the fiber quality of cover crops forages to be used as a partial replacement of whole-plant corn silage (WPCS) in dairy diets in Wisconsin.

Materials and methods

Samples of cover crops used for silage production were collected from multiple dairies in Wisconsin within 60 d of harvest. Samples of triticale ($n = 5$), rye ($n = 6$), ryegrass ($n = 11$), and other cover crops ($n = 11$) from various varieties, agronomic practices, maturities, and silage management were collected. Samples were dried at 60°C for 48 h in a forced-air oven and ground to pass a 1-mm Wiley mill to determine content of neutral detergent fiber (aNDF), in situ undigested NDF at 240 h (uNDF240) and in vitro NDF degradability (NDFD) at 12, 24, 30, 48, and 120 h of incubation. Briefly, the in situ uNDF at 240 h of samples were determined using 0.5 g triplicates in F57 bags incubated in 2 ruminally cannulated Holstein cows. The in vitro NDFD of forage samples were determined using 0.5 g triplicates for each incubation time in F57 bags. These samples were incubated according to Goering and Van Soest (1970). Intake simulations were performed using the equation by Allen et al. (2019). Simulations were conducted to assess the greater limitation of DMI as milk yield increases. Predictions to evaluate the potential effect of partial replacement of corn silage with alternative forages on DMI of lactating cows with a milk yield of 40 (DMI₄₀) and 60 (DMI₆₀) kg/d were observed. Diets were formulated to have the same forage-to-concentrate ratio, regardless of the chemical composition of the cover crops forages. Corn silage-based diet was partially replaced (10% DM) with rye, ryegrass, triticale or a mixture of alternative forages. Data were analyzed as a completely randomized design with the fix effect of forage type, and significance was declared at $P \leq 0.05$.

Results

No differences were observed (Table 1) in the fiber quality variables evaluated in this study between the cover crop forages. These fiber degradability values allowed for the substitution of 10% of corn silage in our simulations, maintaining or increasing milk and milk fat production for dairy cows producing 40 kg and 60 kg of milk per day (Table 2).

Conclusions

Cover crops may partially replace corn silage utilization in Wisconsin dairy farms with the aim of stretching forage inventories, while maintaining predicted milk and milk fat production. However, further in vivo studies are warranted to confirm these findings. Besides, to obtain high-quality silage, producers must account for many factors including logistic and agronomic factors beyond the scope of this study. Selecting good varieties, defining the proper harvest maturity and implementing proper silo management is of utmost importance and further research is warranted.

Table 4. Description of the fiber quality of cover crops used as forage for dairy cattle in Wisconsin.

Item	Triticale	Rye	Ryegrass	Others	SEM	P-value
aNDF, % DM	41.2	52.3	46.3	45.4	3.81	0.43
uNDF, % DM	16.4	20.0	13.6	15.1	1.64	0.11
uNDF, % NDF	32.5	37.3	29.5	33.2	3.06	0.40
12h NDFD, % NDF	19.1	18.0	21.2	22.4	2.33	0.58
24h NDFD, % NDF	34.0	30.7	39.1	44.7	4.19	0.14
30h NDFD, % NDF	46.0	41.0	48.7	52.8	4.21	0.31
48h NDFD, % NDF	57.8	56.5	64.1	62.6	4.14	0.56
120h NDFD, % NDF	70.0	71.2	77.1	75.0	3.35	0.48
pdNDF, % DM	37.3	38.8	35.1	32.8	2.22	0.30
pdNDF, % NDF	72.0	72.5	75.7	72.6	2.45	0.67
Fast Pool, % NDF	57.8	57.3	64.1	62.6	4.29	0.66
Slow Pool, % NDF	14.2	15.2	20.5	14.9	3.86	0.64

¹aNDF: Neutral detergent fiber; uNDF240: undigested neutral detergent fiber at 240 h of incubation; pdNDF: potential degradable neutral detergent fiber; NDFD: neutral detergent fiber degradability

Table 5. Predicted-allowable milk as affected by partial replacement of corn silage with cover crops forages.¹

Item ²	Corn silage-based diet	Triticale	Rye	Ryegrass	Others
Predicted DMI ₄₀					
NEL Allowable, kg/day	38.93	38.17	39.15	38.35	38.16
MP Allowable, kg/day	26.40	28.55	27.71	29.13	27.64
Milk Production, kg/day	32.40	32.50	32.70	32.60	32.30
Milk True Protein, kg/day	0.98	0.98	1.00	0.99	0.98
Milk Fat, kg/day	1.16	1.22	1.17	1.23	1.19
Predicted DMI ₆₀					
NEL Allowable, kg/day	48.39	47.52	48.77	47.87	47.64
MP Allowable, kg/day	30.46	32.97	32.07	33.71	31.97
Milk Production, kg/day	36.70	36.80	37.10	37.10	36.70
Milk True Protein, kg/day	1.14	1.14	1.17	1.15	1.14
Milk Fat, kg/day	1.31	1.38	1.32	1.39	1.35

¹Predictions performed with the NASEM (2021) model.

²Predicted DMI at 40 (DMI₄₀) and 60 (DMI₆₀) kg of milk/d.

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Correlation Analysis in Chemical Composition and Antioxidant Properties of Whole Corn Silage Under Different Additives

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The antioxidant activity of whole corn silage (WCS) is one of the main factors affecting the nutrient retention. In this study, the conventional nutrient composition and antioxidant indices and their correlation of WCS were determined. The results showed that the addition of tea polyphenols reduced the NDF and ADF contents of silage. CAT, SOD, DPPH and FRAP were higher in M and T than in CK. Correlation analysis showed that DPPH was significantly positively correlated with starch and O₂⁻ radical scavenging rates ($P<0.001$). The purpose of this study was to explore the effect of additives on the quality preservation of WCS, and to screening for suitable antioxidants for silage storage.

Keywords: Additives, chemical composition, antioxidant properties, correlation analysis

Introduction

Whole corn silage is playing an increasingly important role in animal husbandry (Ünlü, et al., 2024). Silage additives rich in antioxidants can enhance the antioxidant activity of silage, livestock and their products (Zhang, et al., 2024), it also can extend the shelf life of silage and improve the safety and nutrition of WCS. As a natural preservative, melatonin(M) can effectively scavenge reactive oxygen species and inhibit peroxidation (Li, et al., 2021). Tea polyphenols(T) as natural plant-derived additives, can inhibit the oxidation of fatty acids and also help prevent protein hydrolysis in silage. The purpose of this study was to explore the effect of antioxidants on the quality and safe preservation of WCS by analyzing the chemical composition and antioxidant capacity, so as to provide a theoretical basis for the optimization of additive products for silage storage.

Materials and methods

The silage corn(Jingke Silage 932) used in Tianyuan Sheep Farm of Hongsibao of Ningxia Hui Nationality. After harvesting, raw material (RM) is taken to 3L silage tank (750kg/m³) for 60d. Additive treatment includes: no additives (CK); Melatonin (M): 0.3% addition and Tea polyphenols(T): 0.3% addition. 1.Chemical composition: The pH and NH₃-N content were determined in liquid samples. The dried samples were used to determine crude protein (CP), water soluble carbohydrate (WSC), starch (ST), neutral detergent fiber (NDF) and acid detergent fiber (ADF). 2.Antioxidant enzymes and free radical scavenging rate: The liquid supernatant was used for the determination of catalase(CAT), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and lipoxygenase (LOX) spectrophotometrically using commercially. Lyophilised samples was used for the determination DPPH, ABTS, FRAP, O₂⁻, OH⁻ and H₂O₂ content. available assay kits (Suzhou Keming Bioengineering Institute, China) according to the manufacturer's instructions.

Results

1. Effect of additives on chemical composition of WCS

Table 1. Chemical composition of WCS in different additives

Treatment	pH value	AN(%TN)	WSC(%DM)	CP(%DM)	ST(%DM)	NDF(%DM)	ADF(%DM)
RM	—	—	2.36±0.02a	7.87±0.33a	39.22±1.03a	40.50±0.67a	19.92±0.30a
CK	3.77±0.04a	0.29±0.01b	1.03±0.03b	8.18±0.39a	31.08±1.09c	38.45±0.85ab	19.97±0.63a
M	3.70±0.01a	0.50±0.03a	0.91±0.07b	6.33±0.15b	35.17±0.65b	37.70±1.25ab	18.87±0.29ab
T	3.71±0.01a	0.31±0.02b	1.07±0.08b	6.48±0.16b	34.29±0.96b	36.01±0.90b	17.63±0.95b
P Value	0.108	0.001	0.000	0.003	0.002	0.054	0.078

Note: Lowercase letters indicate differences between treatments $P<0.05$, the same as below.

The pH value was about 3.7, the NH₃-N content produced by M was significantly higher than T and CK. The contents of WSC, CP, ST, NDF and ADF were significantly decreased under the addition of M and T. The NDF and ADF of T were lower than M.

2. Effect of additives on antioxidant enzyme and *In vitro* antioxidant activity in WCS

Table 2. Antioxidant enzyme and *In vitro* antioxidant activity of WCS in different additives

Treatment	CAT (nmol/min/g)	MDA(μmol/kg)	SOD(U/g)	DPPH(μmol Trolox/g DM)	ABTS(μmol Trolox/g DM)	FRAP(μmol Trolox/g DM)
RM	3.86±0.13c	0.08±0.01b	469.19±2.41a	0.63±0.01c	1.15±0.01b	2.62±0.01d
CK	19.08±3.14b	714.67±21.07a	348.43±20.34b	5.56±0.40b	86.97±1.62a	7.37±0.35c
M	26.18±2.19a	670.24±33.90a	384.82±45.55ab	6.97±0.38a	84.76±4.02a	11.02±0.96a
T	17.06±0.89b	649.29±9.94a	409.23±18.31ab	7.16±0.18a	83.68±1.92a	9.31±0.40b
PValue	0.000	0.000	0.141	0.000	0.000	0.000

As can be seen from Tab 2., the CAT, SOD of M and T were higher than those of CK. the DPPH and FRAP contents of M and T were higher than those of CK.

3. Effect of additives on correlation analysis in WCS

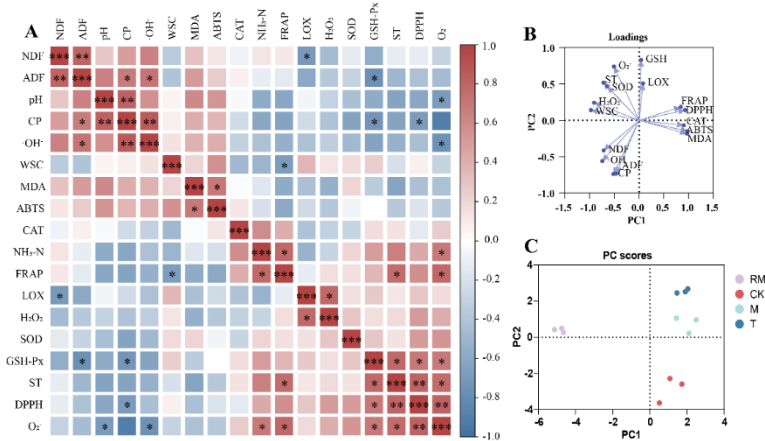


Figure 1. Correlation analysis in WCS

AS the picture shows that samples can be divided into 4 groups(Fig1.C); DPPH, FRAP, ABTS, CAT, MDA have similar effects, and O₂⁻, ST, SOD, H₂O₂, and WSC have similar effects (Fig.1B); DPPH was significantly positively correlated with ST and O₂⁻ radical scavenging (P<0.001)(Fig1.A).

Conclusions

It was found that the chemical composition and antioxidant activity of WCS has significant correlation; the addition of 0.3% tea polyphenols could significantly improve the antioxidant activity of WCS and ensure the nutritional value of the silage.

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Effect of the Inoculant and Grass Baleage Dry Matter on Fermentative and Nutritional Profiles

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Keywords: *Lentilactobacillus buchneri*, *Lentilactobacillus hilgardii*, round bales, silage quality

Introduction

Silage baling is an attractive preservation technique that offers flexibility to the farmer at the time of feeding his herd, while managing better the weather damage risk at the time of harvesting the forage (Coblentz and Akins, 2018). However, the recommended low moisture level (45-55%), together with the longer particles than in “classical” bunker silos poses other critical issues of a more limited fermentation and potential higher sanitary risk due to the development of undesirable microorganisms (*Clostridium*, *Listeria*, fungi). Inoculants represent a helpful tool to better manage silage fermentation especially when DM content of the harvested material increases (Guo et al., 2013; Santos et al., 2014), however very limited references are available to assess the dose of inoculant to the dry matter (DM) content of the roughage. Knowing the slower fermentation when DM rises, the increase of the inoculation application rate may become a relevant practice to further secure a good fermentation in difficult-to-ensile material. Grass baleage falls into this situation and the opportunity to compare two doses of inoculant according to the grass DM set the rationale of this study.

Materials and methods

In 2022, a field of 50% fescue (*Festuca arundinacea*, var. Tower) and 50% cocksfoot (*Dactylis glomerata*, var. Lucullus) herbage has been mowed around boot stage and wilted for 24 or 48h before baling. Water (CON) or an inoculant (INO) containing *Lentilactobacillus hilgardii* CNCM-I-4785 (75,000 cfu/g of fresh forage), *Lentilactobacillus buchneri* NCIMB 40788 (75,000 cfu/g of fresh forage), *Pediococcus pentosaceus* NCIMB 12455 (100,000 cfu/g of fresh forage), beta-glucanase (4945 DNS/g), and xylanase (12,600 DNS/g) was applied at 1X (24h) or 1.5X (48h) wilting. Twenty bales were prepared at low DM (35.6%) and eighteen bales at high DM (42.3%). Although CON bales were harvested first, every other windrow was collected in the field to keep the same grass diversity across the field within each treatment.

Each round bale was weighted after wrapping and 90d later. DM was determined at the field level for the day of baling and on an individual basis at opening. A proximate analysis (NIR) was performed on the fresh forage and at the end of the fermentation for crude protein (CP), NH₃-N, total sugar, NDF, ADF, ADL, ash, organic matter digestibility (OMD) and Net energy of lactation was calculated. Fermentation parameters were determined by NIR after 90d of storage: pH, acetic acid, lactic acid, ammonia nitrogen (NH₃-N) and alcohols (ethanol and 1,2-propanediol (1,2-PPD)). Total yeast, moulds and spores counts of *Clostridium tyrobutyricum* were reported at opening too. Data was analyzed within each level of DM using a one-way Anova since the effect of the dose of inoculant and the DM at harvest were confounded (RStudio). The bale was the experimental unit. Statistical significance was defined as $P \leq 0.050$.

Results

After 90d of storage, bales DM increased in both treatment groups by circa 10 points regardless of the initial DM. Fermentation has been properly driven in inoculated bales since acidification was better (reduced pH, increased lactic acid, Table 1), illustrating a dominant homofermentative activity. However, the higher concentration of acetic acid and 1,2-propanediol with INO supports the active contribution of the two heterofermentative inoculated strains, following an efficient pattern due to a lower ethanol content. A direct effect of these bacteria activity is the mitigation ($P < 0.01$) of the yeast growth, for both DM, even if less pronounced with high DM bales. Moulds were below the detection limit. Spores of *C. tyrobutyricum* were halved in low DM grass silage while not impacted in high DM bales. Fermentation losses were only reduced in high DM bales with INO on a fresh matter basis (1.19 vs 2.04% FM).

Table 6. Fermentation profile of grass silage bales according to the inoculation and the DM at baling

	Low DM				High DM			
	CON	INO	SEM	P-value	CON	INO	SEM	P-value
DM, g/kg	453.3	450.7	14.0	0.855	508.5	502.6	20.6	0.778
pH	5.63	4.58	0.08	<0.01	5.81	4.69	0.09	<0.01
Lactic ac., g/kg DM	12.8	40.4	3.5	<0.01	9.5	31.5	2.5	<0.01
Acetic ac., g/kg DM	5.2	27.8	2.0	<0.01	4.2	15.3	1.5	<0.01
NH ₃ -N, % N	8.9	8.5	0.4	0.273	7.6	8.1	0.6	0.477
Ethanol, g/kg DM	28.4	6.2	4.6	<0.01	24.8	5.7	4.0	<0.01
1,2-PPD, g/kg DM	0.3	19.8	1.1	<0.01	0.2	8.0	1.2	<0.01
Yeasts, Log CFU/g	5.99	2.04	0.32	<0.01	5.52	3.29	0.77	0.011
Spores, Log count/g	1.63	0.87	0.17	<0.01	0.75	1.00	0.12	0.158

The more intense fermentation reflects into a reduced amount of sugars in INO bales, while an improved nutritional profile is depicted in the low DM bales notably: higher net energy value (UFLx1760 kcal), more intestinal digestible

protein (PDI) leading to a greater rumen protein balance (Table 2). The reduced value for rumen fill supports more dry matter voluntary intake, starting point of a greater nutrients supply. INO impacted more the nutritional value of low DM bales but PDI was consistently increased at both DM.

Table 7. Nutritional profile of grass silage bales according to the inoculation and DM at baling

	Low DM				High DM			
	CON	INO	SEM	P-value	CON	INO	SEM	P-value
OMd, %	73.06	73.97	0.26	<0.01	72.87	73.44	0.40	0.177
Sugar, g/kg DM	60.80	39.70	6.75	<0.01	67.75	44.20	7.19	<0.01
Crude prot., g/kg DM	128.80	135.30	2.60	0.023	136.95	141.40	4.24	0.311
Ash, g/kg DM	95.9	92.0	1.57	0.024	96.54	97.76	1.74	0.492
NDF, g/kg DM	583.60	564.9	4.66	<0.01	581.44	567.33	4.76	0.010
UFL*, /kg DM	0.90	0.92	0.004	<0.01	0.89	0.90	0.004	0.174
PDI*, g/kg DM	72.18	73.77	0.50	0.038	75.51	78.13	0.82	0.038
RPB*, g/kg DM	3.25	9.75	1.48	<0.01	8.02	10.69	2.34	0.431
Rumen fill, u/kg DM	1.106	1.079	0.002	<0.01	1.097	1.092	0.003	0.258

* UFL: Forage unit milk; PDI: proteins digestible in the intestine; RPB: rumen protein balance

Conclusions

The increase of the dose of inoculation with the DM at baling looks adequate since fermentation was improved in both scenarios, besides less favorable conditions when DM increases. However, the accurate characterization of DM losses was not conclusive enough, likely due to the variability around bales weighing and samples for DM determination. The greater amount of 1,2-propanediol and acetic acid in the inoculated bales certainly contributed to limiting yeast growth as a marker of improved quality of the preserved forage. Aligned with the enhanced fermentative profile, nutritional value of the low dry matter baleage was improved while the same numerical trend was observed in the high dry matter material. Finally, the reduced presence of butyric spores in the low DM bales further illustrates a beneficial effect of this inoculant at mitigating the hygienic risk associated with grass silage bales.

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Effects of a Postbiotic Additive on Anaerobic Fermentation of Corn and Alfalfa Silage Liquid Media

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Keywords: post-biotic additive, silage liquid media, fermentation

Introduction

Improving silage fermentation after harvest is important for a healthy diet in dairy production systems. A variety of additives are added to silage with the aim of spatial or temporal improvement of silage fermentation. Depending on the constituents of the additive, fermentation could be aided or impeded. There are a number of commercial products that claim to accelerate fermentation or increase lactate when added to silage. In this study, we evaluate the in-vitro efficiency of a postbiotic fermentate containing a mixture of yeast cultures and the media on which they were grown on liquid extracts of brown midrib (BMR) corn (*Zea mays*) and alfalfa (*Medicago sativa*) silage.

Materials and methods

A three-by-four-by-four factorial in-vitro experiment was conducted in 16x150mm glass culture tubes. Juice from 100g of corn, alfalfa was obtained by pressing with a 6-ton press. 1mL of juice or distilled water (control) was mixed with postbiotic additive (Sweetpro Probiotein®, Harvest Fuels, Walhalla, ND, USA) at four dosages on a dry matter basis: control-0g, low-0.1g, medium-0.5g, & high-1g. Additive and juice/water mixtures were transferred to sterile culture tubes containing 10 ml 50% MRS broth in quadruplicate and purged with dinitrogen gas to remove oxygen. Tubes were sealed with butyl rubber stoppers and incubated anaerobically for 48 hrs at 30°C while shaking at 150 revolutions per minute (rpm). After 48 hrs, pH was measured, and 1 ml of liquid from each test tube was analyzed for concentration of fermentation products via high-performance liquid chromatography (HPLC). Linear model and analysis of variance (ANOVA) were conducted in R.

Results

Data analysis revealed a significant ($P < 0.05$) plant-by-dose interaction effect on lactate concentration. For both corn and alfalfa media, lactate concentration from low dosages of additive was not different from the controls without additive. However, high dosages of the additive in alfalfa and corn media yielded the highest (189mM & 118.8mM respectively) lactate concentration for each plant juice. pH was consistent (~3.9) across extracts from both alfalfa and corn. However, distilled water tubes with additive averaged pH 4.8, and the distilled water control without any additive was at 6.36. A significant ($P < 0.05$) main effect of the additive was detected on succinate concentration with the high dosage of additive yielding the highest concentration (2.13mM). There was no main effect of dosage on acetate and ethanol concentration, but there was a significant ($P < 0.05$) effect of plant juice on acetate and ethanol concentration. Juices obtained from corn yielded significantly more ethanol and acetate with a difference of 33.6mM and 22mM, respectively, compared to alfalfa.

Conclusions

Addition of a postbiotic additive improved lactate and succinate concentrations in in-vitro silage studies. These results indicate potential for the use of postbiotic additives to improve forage fermentation and subsequent work should investigate performance during ensiling.

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Integrated Management of Vomitoxin in Corn Silage Fields and the Fate of Vomitoxin During Ensiling

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Keywords: vomitoxin, feed safety, ensiling

Introduction

Integrated management involves using a variety of strategies to manage diseases and toxins without exclusive dependence on chemical sprays. Decisions about corn (*Zea mays*) hybrids and how they affect toxins when growing corn for silage are essential considerations for maximizing sustainable production. Toxins within the corn silage production pipeline from the field to the bunker silos negatively impact feed quality for dairy animals. Therefore, in-season and post-harvest management of mycotoxins is crucial. However, little is known about the accumulation of toxins in silage corn hybrids ensiled over a long period. The toxin being investigated for this study is deoxynivalenol (DON), also known as vomitoxin, produced by *Fusarium graminearum* during the infection of corn hybrids. Brown midrib (BMR) hybrids have gained popularity in silage corn production because of their low lignin content and high digestibility. Conversely, conventional or dual-purpose hybrids offered unique benefits like disease resistance. The impact of fungicides on toxins in BMR hybrids has been studied, but little is known about how BMR hybrids compare to dual-purpose hybrids. In addition, it is also not known how toxin accumulation occurs across time in both hybrids and what happens to the toxin during ensiling.

Materials and methods

To answer these questions, we conducted multi-year field trials in Arlington, Wisconsin, in 2020 and 2021. For the first set of trials, we evaluated two corn hybrids, six fungicide application regimes, and a non-treated control for in-season management of toxins. We compared the concentration of the toxin, deoxynivalenol (vomitoxin) in a BMR and a conventional hybrid. For the second trial, two fungicide application regimes and a non-treated control were evaluated for their effect on the two hybrid classes during ensiling. After harvest, we simulated ensiling by incubating samples of chopped silage across 5 different time points (Harvest, 0, 30, 60, 90, 120) days. Samples from each time point were evaluated for moisture, pH, digestibility parameters, and vomitoxin concentration.

Results

Our results and analyses show a significant ($P < 0.01$) main effect of hybrid on the yield, silage quality, and total vomitoxin observed at harvest. The BMR hybrid class had higher vomitoxin concentration and better digestibility parameters. Fungicide application regimes during the growing season marginally influenced vomitoxin concentration but strongly impacted tarspot disease severity. During ensiling, we observed significant ($P < 0.01$) main effects of hybrid and ensiling duration on vomitoxin. BMR hybrid had higher vomitoxin concentration across treatments and ensiling duration. While analysis of the effect of ensiling duration showed a significant ($P < 0.01$) difference in vomitoxin during the first time interval. Vomitoxin concentration was higher after 30 days of ensiling compared to levels at harvest (Table 1).

Table 1. Main effect of time on pH and the concentration of vomitoxin also known as deoxynivalenol (DON) from two corn hybrids (Brevant® brand BMR hybrid, B10B77SX™, and a Brevant® brand dual-purpose hybrid, B08J81AMXT™) treated with Headline AMP®; active ingredient (a.i.), pyraclostrobin (0.15 kg/liter a.i.) + metconazole (0.06 kg/liter a.i.), Proline®; a.i., prothioconazole (0.48 kg/liter a.i.), or not treated and chopped for corn silage and ensiled at 30 days interval for 120 days. Data are from field experiments conducted in 2020 and 2021 and pooled together for analysis.

Ensiling Duration [†]	0 (Harvest)	30 days	60 days	90 days	120 days	P-value
pH	5.86a	3.81b	3.80b	3.78c	3.86bc	< 0.01
DON (ppm)	0.85a	1.24bc	1.34b	1.48bc	1.42b	< 0.01
Moisture (%)	63.78c	65.53a	64.92ab	64.55b	64.57abc	< 0.01

[†] Means across a row (time-point comparison) not followed by the same letter are different ($P < 0.05$).

Conclusions

Our findings suggest that lower vomitoxin (DON) levels could be achieved by using conventional hybrids. Fungicide applications during the growing season were not consistently effective in reducing toxin concentration observed at harvest. We also show that there is a significant increase in the vomitoxin concentration observed between harvest and 30 days later. The hybrid of corn used and the concentration of toxins at harvest contribute to determining toxin concentration after ensiling for 30 days. After 30 days of ensiling, vomitoxin levels remain relatively stable. Growers are advised to use more toxin-tolerant hybrids and test for toxins after 30 days to be certain of the actual toxin levels at feed out.

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Postbiotic Additive Effects on Fermentation and Forage Quality of Corn and Alfalfa Silage During Ensiling

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Keywords: additive, corn silage, silage improvement

Introduction

Silage additives are used to improve silage fermentation or quality. The postbiotic yeast fermentate additive evaluated here was shown to enhance the quality of fermentation parameters *in vitro*. This study further evaluates the efficacy of postbiotic additive doses on the fermentation and quality of silage after chopping and during ensiling, specifically focusing on corn and alfalfa as the types of silage being assessed for their performance under the influence of these additives.

Materials and methods

Three doses, control - 0%, low - 0.05%, & high - 0.5% (w/w dry matter basis), were evaluated on whole crop second-cut alfalfa and brown midrib (BMR) corn. Prepared samples were ensiled in four replicate 300g mini silo bags per treatment and vacuum sealed. Mini silos were incubated after sealing at 30 °C for 0, 3, 7, 21, and 60 days. Following ensiling, sample bags were opened and analyzed for pH, dry matter loss, fermentation products, and digestibility parameters. Data obtained were analyzed in R using relevant packages.

Results

In both crops, lactate and acetate concentrations consistently increased over time. However, alfalfa samples produced significantly more lactate than corn. The significant ($P < 0.05$) plant-by-dosage interaction effect revealed no differences among corn treatments and lower lactate concentration when the high additive rate was applied to alfalfa. Irrespective of additive dosage, alfalfa samples had higher lactate concentrations than corn. The trend for acetate was identical. In contrast, all the doses added to corn yielded statistically similar acetate concentrations, and there was a significant dosage-by-time interaction effect. There was a significant ($P < 0.05$) plant-by-dosage-by-time interaction effect on the pH and loss of plant dry matter during ensiling. Corn silage ensiled for 21 days had the lowest pH (3.3) across all doses, while alfalfa at harvest had the highest pH (5.9) across all doses. Dry matter reduced sequentially during ensiling with the most loss (-18.6g) in corn silage samples with high additive dose and ensiled for 60 days, followed by alfalfa (-14g) ensiled for 60 days irrespective of additive.

Conclusions

The additive contributed minimally to overall silage and fermentation quality parameters. Most of the differences observed were driven by crop type and the duration of ensiling. Results suggest that the evaluated additive is capable of supporting fermentation, but higher doses may be inconsequential or deleterious to overall silage quality. Subsequent work should evaluate optimal dosing as well as earlier ensiling times and in combination with silage inoculants.

References

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Selective Inoculations Using a Precise Combined Harvesting Machine

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Keywords: precision farming, combined harvesting machine, heterofermentative.

Introduction. Quality indexes (Qi) provide a single numerical representation of the overall quality of maize silage, with aerobic stability (AS) being a critical factor during the feed-out phase. Various factors during silage preparation, such as harvested maize composition [1], ensiling conditions and procedures, or additives, can influence the Qi value and the AS of maize silage. Our study focused on pre-ensiling conditions that affect Qi and AS, aiming to use these outcomes to enable optimal management decisions during ensiling automatically. Drawing on principles of precision agriculture and previous work (Patent WO2019/243615 A1), we proposed the use of sensors integrated into harvester machines to monitor key maize traits in real-time. This approach would facilitate informed decisions, including selecting appropriate heterofermentative inoculant dosage to optimize silage quality and aerobic stability.

Material and Methods. Fresh whole maize plants (FWMP) were ensiled using two mixtures of obligate heterofermentative *Lentilactobacillus buchneri* (Lb), specifically KWS LACTOSTABILITY (AGRAVIS Raiffeisen AG, Munster, Germany) (CCM 1819), at different concentrations: a standard dose (SD) of 2.02×10^5 CFU/g of fresh forage, a decupled dose (DD) of 2.02×10^6 CFU/g of fresh forage, and a control (pure water, C). To simulate in-field harvest variability, maize was sown in a single field plot divided into six subplots arranged in a checkerboard pattern with three hybrids KWS Simpatico (200 FAO class), KWS Adaptico (300 FAO class), and KWS Inteligens (400 FAO class). The maize was sown on April 6, 2023, and harvested on August 4, 2024, in the Veneto region (northeast Italy). During harvesting, the harvesting machine employed a precision NIR to determine the composition of FWMP and adjust the dosage accordingly within a range of 80% to 150% (Patent WO2019/242615 A1) for SD, DD, and C. All three maize hybrids were harvested equally to maintain consistency across the SD, DD, and C harvested FWMP variability. FWMP were then ensiled into bale silage (11 repetitions per thesis) of approximately 1 m³ using a VARIO-Master V140 (Göweil Maschinenbau GmbH, Kirchschlag, Austria). After 60 days, maize silage samples were analyzed for chemical composition. Three repetitions for each inoculant were ensiled in 10-L silage buckets and, after 60 days, were exposed to air to assess the AS. The temperature of the silage was measured using dataloggers (Elitech USB Temperature Datalogger RC-5, London, UK). The AS was defined as the duration (in hours) for which the silage remained within 2°C of room temperature while exposed to air.

Results

Harvested FWMP differs in dry matter (DM, %) and content for the three inoculants (28.3b, 28.2b, and 30.3a— $P < 0.0001$ —for SD, DD, and C, respectively), but water-soluble carbohydrates (WSC, % DM) did not differ among thesis (7.04, 7.03, and 7.81 for SD, DD, and C, respectively). Due to the high moisture and WSC content, the FWMP profile corresponds with a highly fermentable substrate. The DM density (146 Kg DM/m³) did not differ in the three theses but did not satisfy the recommended threshold of 240 Kg DM/m³ to limit the porosity at 0.40 [2]. The observed porosity was 0.57b, 0.56b, and 0.59a ($P = 0.001$) for SD, DD, and C, respectively, exposing the silage to aerobic deterioration risk. Differences in Qi pH, mannitol, ethanol, lactic and acetic acids were observed in the three theses (Table 1), denoting a better fermentative profile in SD and DD, compared C.

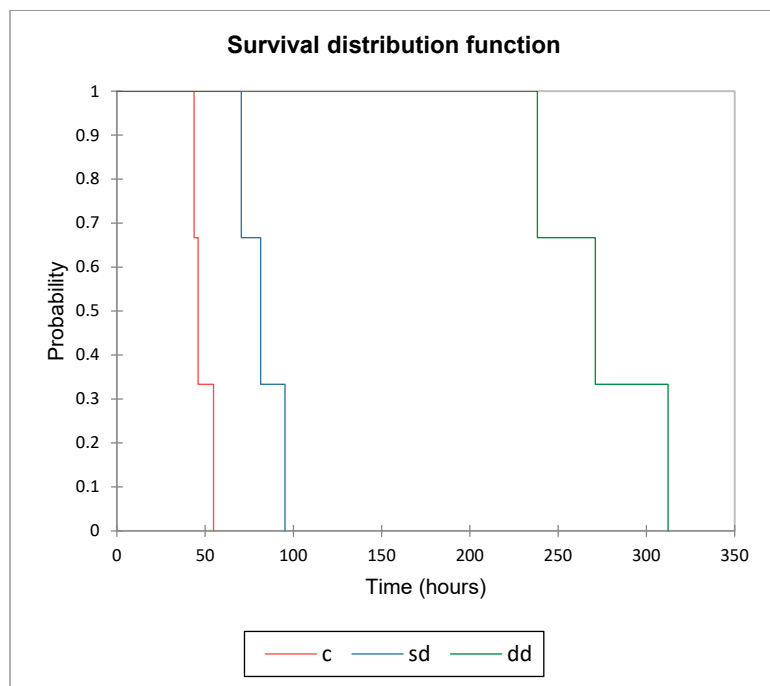
Table 1: the quality index (Qi), and the maize silage's fermentative profile underwent three inoculations. C = pure water, SD = standard dose; DD = decupled dose. Whitin constituent the letters indicate differences in means with $P = 0.05$.

	Qi (0-100)	pH	Ammonia (NH4/N, %)	Mannitol (DM)	(% Ethanol (% DM)	Lactic ac. (% DM)	Acetic ac. (% DM)	Propionic ac. (% DM)	Butiryc ac. (% DM)
C	42.1b	3.87	6.12	0.21b	0.93a	4.24b	1.90	0.82	0.08
SD	56.4a	3.84	6.38	0.50a	0.64b	5.25a	1.97	0.82	0.08
DD	52.1a	3.85	6.28	0.63a	0.62b	4.79a	1.86	0.83	0.08
P	<0.0001	0.053	0.393	0.001	<0.0001	<0.0001	0.664	0.964	0.134

However, after seven days of aerobic exposure, the lactic (5.39a, 5.44a, and 2.00b— $P = 0.001$ —, for SD, DD, and C, respectively) and the acetic acids (1.00a, 1.55a, and 0.23b— $P = 0.002$ —, for SD, DD, and C, respectively) remains higher in the inoculated maize silage, compared to the control, denoting a prolonged effect of the additive in the fermentative pattern.

At the survival analysis, the Kaplan-Meier (Figure 1) revealed that aerobic stability significantly differs for the three theses (Log-rank test $P = 0.025$). The mean survival times were 82.4b, 274a, and 48.8b ($P < 0.0001$) for SD, DD, and C, respectively, denoting a manful effect of a reinforced dose of inoculants. However, the aerobic stability time for the SD is compatible with feed-out rates in most commercial farms.

Figure 1: The Survival distribution function for the maize inoculated with pure water (C), the standard dose (SD), or the decupled dose (DD). The time expresses the hours of aerobic stability of maize silage exposed to air, while the probability expresses the survival rate at



Conclusions

A precise inoculation system based on the harvested maize composition enabled us to achieve more stable silage during the feed-out phase compared to using a control. This stability is likely attributed to the sustained presence of lactic and acetic acid content when the inoculated silage is exposed to air. Although the standard dose of inoculant is suitable for commercial farm standards, using a higher dose could be more beneficial. However, the cost of the inoculants prohibits the use of the maximum decupled dose, which represents an extreme scenario within the dosage range. Therefore, the results suggest the need to fine-tune the dosage to minimize the risks of silage degradation while optimizing the cost-effectiveness of inoculations.

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REDUCING CORN SILAGE LOSSES AND IMPROVING AEROBIC STABILITY UNDER ADVERSE CONDITIONS: FARM-SCALE TRIAL IN ARGENTINA

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Keywords: dry matter loss, aerobic stability, microbial inoculant, corn silage

Introduction

High-quality forage is essential for feeding high-yielding dairy cows, with whole-plant corn silage playing a key role in dairy diets in Argentina. Proper conservation and stability during storage and feed-out are crucial to minimize losses and maintain nutritional value of the ensiled material. Adverse conditions, such as weather stress, and pathogens like *Spiroplasma*, pose significant challenges to silage preservation (Jones & Medina, 2020). *Spiroplasma* transmitted to plants by insect vector, can impair corn development, reduce starch accumulation, and compromise silage fermentation and aerobic stability. Selecting the adequate inoculant with specific microorganisms and concentrations is essential to enhance silage fermentation, reduce dry matter losses, and control temperature fluctuations during feed-out (Muck et al. 2018). In this study, we evaluated the effect of two bacterial inoculants on whole-plant corn silage affected by *Spiroplasma*, focusing on dry matter losses and temperature dynamics.

Materials and methods

The trial was conducted at a commercial dairy farm (Chiavassa Group) in Carlos Pellegrini, Santa Fe, Argentina, using corn affected by *Spiroplasma*, resulting in a dry matter (DM) content of 52 ± 2.0 %. The corn was harvested between April 26th and 28th, 2024. Fifteen silobags (~ 290 tons each) were produced and divided into three treatments: control treatment without inoculant (CON, n= 5); inoculant 1 (a blend of *Lactobacillus curvatus*, *Lactobacillus lactis*, *Pediococcus acidilactici*, *Enterococcus faecium*, *Propionibacterium acidipropionici*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus buchneri*, and cellulolytic enzymes) applied at 160,000 CFU/g of fresh matter (ST, n= 5) inoculant 2 (*Lactobacillus lactis* DSM11037 and *Lentilactobacillus buchneri* DSM22501; SILOSOLVE® FC, Novonesis, Lyngby, Denmark) applied at 150,000 CFU/g of fresh matter (FC, n= 5). After 30 and 60 days of ensiling, samples were collected to assess DM losses and temperature dynamics. Following sample collection, the silobags were resealed at each respective time point. Three subsamples from each silobag were combined to form a composite sample (~ 2.5 kg) and placed in 10-liter open buckets (n= 5 per treatments). A datalogger was installed in each bucket to monitor temperature, and samples were exposed to oxygen for 10 days under ambient conditions. Data were analyzed as a completely randomized design using the MIXED procedure of SAS. Buckets were weighed and sampled at the start and end of the 10-day aerobic exposure period to determine DM losses. Each silobag served as the experimental unit for statistical analysis, with significance set at $P \leq 0.05$.

Results

At 30 days post-harvest and following 10 days of exposure to oxygen, DM losses in the FC treatment (0.4 %) were significantly lower ($P < 0.05$) compared with ST (3.9 %) and CON (5.6 %), while no significant differences between ST and CON could be detected ($P > 0.05$). Additionally, the maximum temperature recorded in the FC treatment (22.3 °C) was significantly lower ($P < 0.05$) than ST (25 °C) and CON (24.3 °C) treatments, while no significant differences between ST and CON were observed ($P > 0.05$). Temperature variations were not significantly different among treatments ($P > 0.05$). The average ambient temperature during the 10-d aerobic challenge was 17.5 °C. At 60 days post-harvest, differences in DM losses ($P < 0.05$) were recorded among all treatments with FC showing the lowest (1.8 %), then CON (3.4 %) and ST at the highest level of DM loss (6.04 %). No significant differences were observed in the maximum temperature between the CON (15.5 °C) and FC (15.9 °C) treatments, while the ST treatment (16.8 °C) expressed a statistically significant higher maximum temperature ($P < 0.05$). The average ambient temperature during this period was 12.5 °C.

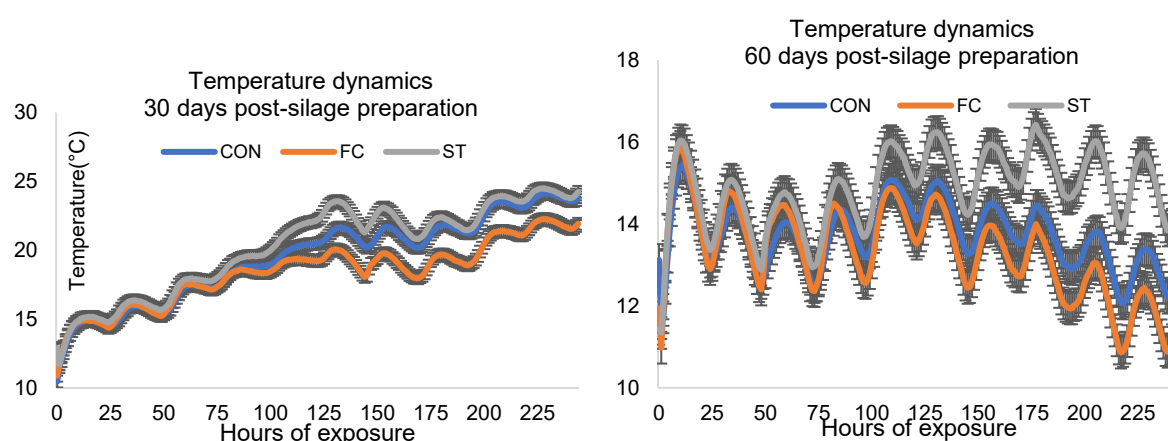
Table 1: Dry matter loss during 10 days of aerobic exposure and maximum temperature °C of the silages after 30 and 60 days of fermentation.

Item	Treatments			SEM	P value
	CON	FC	ST		
Day 30					
Dry matter, %	53.8 ^a	51.9 ^b	49.0 ^c	0.51	< 0.0001
Dry matter loss, %	5.6 ^a	0.4 ^b	3.9 ^a	0.665	< 0.001
Maximum temperature, °C	24.3 ^a	22.3 ^b	25.0 ^a	0.60	0.02
Day 60					
Dry matter, %	51.0 ^a	52.2 ^a	48.5 ^b	0.64	< 0.01
Dry matter loss, %	3.4 ^b	1.8 ^c	6.04 ^a	1.265	0.04
Maximum temperature, °C	15.5 ^b	15.9 ^b	16.8 ^a	0.29	0.02

Different letters indicate significant differences (P < 0.05)

The temperature dynamics during 10 days of aerobic exposure of samples collected 30 days following fermentation revealed that, from 84 hours onwards, the CON and ST treatments exhibited significantly ($P < 0.05$) higher temperatures, with ST reaching the highest maximum temperature. The FC treatment maintained lower temperatures compared to the two other treatments throughout the 10-day period. For samples collected following 60 days of fermentation, temperature differentiation began at 96 hours, with the ST treatment showing a significant increase in temperature.

Figure 1: Temperature Dynamics During Aerobic Exposure of Silages After 30 and 60 Days of Fermentation



Conclusions

Our results demonstrated that inoculant composition affects DM losses and temperature dynamics during aerobic exposure of whole-plant corn silage affected by *Spiroplasma*. The FC inoculant maintained lower temperatures and reduced DM losses, highlighting its effectiveness in preserving silage quality under adverse conditions.

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Evaluation of Mycotoxins in the Corn Silage Fed to Dairy Cows in Southeast of Brazil

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Keywords: contamination, fungi, bovine, toxicity

Introduction

The contamination of animal feed with multi-mycotoxins represents a significant challenge that affects a wide range of sectors, including the animal production and food industries. Mycotoxins are toxic compounds that are produced as secondary metabolites by the metabolism of fungi and can have a number of adverse effects on the health and performance of animals. The effects of mycotoxins have been observed to include reduced performance, milk production and weight loss. Despite their greater resistance due to rumen metabolism, ruminants are more susceptible to mycotoxin contamination due to their highly diversified diet. In Brazil, the tropical characteristics provide suitable climate conditions for the growth of various species of mycotoxin-producing fungi. The combination of high temperatures and elevated humidity creates an environment that facilitates the proliferation of these organisms, thereby increasing the risk of contamination of the feed with mycotoxins. Thus, objective of this research was to determine and quantify which mycotoxins were present in the corn silage fed to dairy cows in the Southeast region of Brazil. The data presented serve to highlight concerns regarding the co-occurrence of mycotoxins, a phenomenon that is becoming increasingly prevalent.

Materials and methods

A total of hundred eighty corn silage samples were collected from dairy farms situated in the southeastern region of Brazil. In each dairy farm, 500 g of the available corn silage were collected, transported to the laboratory, and subjected to a drying process in a forced ventilation oven at a temperature of 105 °C for 24 h, to determine the dry matter content. Subsequently, the dried corn silage samples were subjected to grinding and reserved for mycotoxin analysis. The analyses of multiple mycotoxins in corn silages: aflatoxins (AF) B1, B2, G1 and G2, deoxynivalenol (DON), fumonisins (FB) B1 and B2, ochratoxin A (OTA), HT-2, toxin T2 (T2) and zearalenone (ZEN) samples were conducted strictly following the methodologies described by Sulyok et al. (2007), and validated previously in the laboratory (Franco et al., 2019) using a Waters Acquity I-Class ultra-performance liquid chromatography (UPLC) system (Waters Corp.) equipped with a BEH Column C18 (2.1 × 50 mm, 1.7 µm) and coupled to a Xevo TQ-S mass spectrometer (Waters Corp., Milford, MA, USA). The chromatographic conditions used were identical to those previously described for feed samples by Borowsky et al. (2024). Mass spectrometry (MS) analyses were conducted in a multiple-reaction-monitoring (MRM) mode by using electrospray ionization in a positive ion mode. Following the identification and quantification of the mycotoxins present in the samples, the means, standard deviations, and corresponding maximum and minimum limits were calculated.

Results

The concentrations of mycotoxins in corn silage (CS) samples are presented in Table 1. OTA and T-2 and HT-2 toxins were not detected in the CS samples. However, AFs (sum of B1, B2, G1 and G2), FBs (sum of FB1 and B2), ZEN, and DON were detected at mean levels ranging from 26.8 to 586.9 µg/kg, with a considerable range of variation, ranging from 4.92 to 12798.2 µg/kg.

Regarding the co-occurrence of mycotoxins in CS, 52, 35 and 9% of the samples analyzed contained two (FB and ZEN), three (FBs, DON and ZEN) or four (AF, DON, FB and ZEN) mycotoxins, respectively, as shown in Figure 1.

Table 1. Mean values, standard error (SE), and minimum and maximum concentrations of mycotoxins in corn silage (n = 180) consumed by dairy cows from the southeastern region of Brazil.

Mycotoxin	n (%)	Mean ± SE (µg/kg)	Minimum (µg/kg)	Maximum (µg/kg)
Aflatoxins (B ₁ + B ₂ + G ₁ + G ₂)	32 (18)	26.8 ± 13.47	4.92	68.4
Deoxynivalenol	46 (25)	236.1 ± 93.65	49.21	2688.1
Fumonisin (B ₁ + B ₂)	180 (100)	586.9 ± 58.92	43.19	12798.2
Zearalenone	161 (92)	92.33 ± 18.08	13.29	813.4

n: number of positive samples.

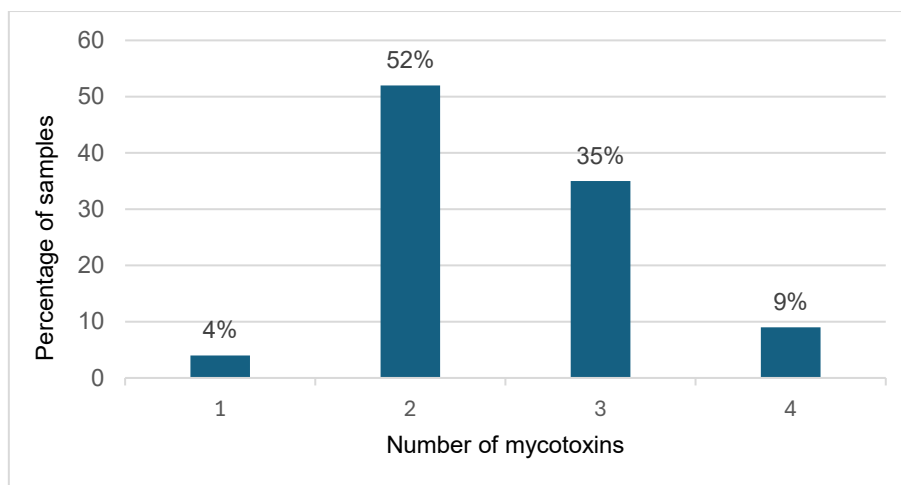


Figure 1. Percentage of samples of corn silage consumed by dairy cows from the southeast region of Brazil containing one, two, three or four mycotoxins.

Conclusions

The findings of this study indicate a possible issue concerning the occurrence of multiple mycotoxins, a phenomenon that is becoming increasingly prevalent. The high prevalence and levels of aflatoxins, fumonisins, deoxynivalenol and zearalenone identified in this research suggest a potential concern regarding the co-occurrence of these mycotoxins. This indicates the necessity for the effective selection of anti-mycotoxin additives capable of acting against the various types of mycotoxins that may be present in corn silage.

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Acknowledgments

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Effect of Laboratory Bag Silo Plastic Type on Fermentation of Corn Silage

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Keywords: organic acids, plastic, yeast.

Introduction

The conventional plastics used to cover bunker and pile silos and wrapped silage bales have relatively high oxygen transmission rates (OTR). Oxygen penetrating silage masses from these plastics can initiate aerobic spoilage from yeasts and spoiled silage leads to a loss of DM and nutritive value and can negatively affect animal performance. Research has documented that using plastics with low OTR results in more desirable silage fermentations with higher recovery of DM, less inedible silage and greater aerobic stability. Our objective was to test a variety of plastics differing in OTR on silage fermentation using laboratory scale silo bags stored for moderate (6 mo) and long (12 mo) periods of time.

Materials and Methods

Experimental silo bags (approximately 100 cm × 20 cm) were made from 8 different plastic sources (Table 1; n = 10 per plastic type) by heat sealing the 2 long perimeter sides and 1 short perimeter side of 2 cut pieces of plastic. Whole-plant corn was harvested at 41% DM using a pull-behind chopper with kernel processor (roller gap setting of 1.40 mm) and chopped to a theoretical length of 19 mm. Experimental silo bags were weighed, filled with approximately 2.5 kg of chopped forage, and air was manually pressed out prior to heat sealing the remaining short perimeter side. Silos were stored in the dark at about 30°C for 6 and 12 mo. Bags were vented as needed during the first 7 d of ensiling by making a small puncture with a sterilized needle, gently expelling the gas, and taping the hole. At the time of silo opening, a portion was used to measure dry matter (DM), and water extracts were used to measure pH, water soluble carbohydrate (WSC), ammonia N (NH₃-N), lactic acid bacteria (LAB), yeasts, and organic acids. The remaining sample was used to measure aerobic stability (AS), defined as the number of hours following air exposure for silage temperature to increase by 2°C.

Results

Plastic types 1 and 2 contained almost entirely mold after 12 mo of ensiling and were not sampled. At 6 mo, silage in plastic types 1 and 2 had the highest concentration of yeasts and LAB, and lower pH and lactic acid than the other silages (Table 2). Both those plastics were very thin and easily penetrated by forage particles. It is likely that small holes provided oxygen to allow spoilage organisms to take hold. Among the remaining plastics, there were only differences in organic acids, with lactic acid being lower and acetic acid being higher for type 7 compared to most other types. Aerobic stability was unaffected by plastic type at 6 mo. Results at 12 mo are presented in Table 3. Silage in plastic type 8 had higher DM and longer aerobic stability than all other silages. Silage in plastic type 7 (standard low-density polyethylene) had higher DM, NH₃-N, LAB, pH, and acetic acid than most other silages, and the lowest aerobic stability. In addition, that silage had 4.0 log yeast while all other silage was below the 2.0 log threshold (data not shown).

Table 1. Description of plastics used in the experiment.

Plastic number	Thickness, μm	Description
1	45	Thin low-density polyethylene
2	45	Thin oxygen barrier plastic
3	45	Thin oxygen barrier plastic
4	45	Thin oxygen barrier plastic
5	45	Thin oxygen barrier plastic
6	80	Thick oxygen barrier
7	125	Standard low-density polyethylene
8	40	Thin oxygen barrier plastic

Table 2. Effect of plastic type on measurements following 6 mo of ensiling.¹

	DM	WSC	NH ₃ -N	Yeasts	LAB	pH	LA	AA
Plastic type								
1	34.16 ^b	6.37 ^{ab}	0.090 ^a	5.96 ^a	8.40 ^a	4.21 ^a	1.96 ^c	2.84 ^{ab}
2	37.11 ^{ab}	4.50 ^b	0.071 ^{ab}	6.78 ^a	8.58 ^a	4.24 ^a	1.70 ^c	2.89 ^{ab}
3	38.08 ^{ab}	7.62 ^{ab}	0.068 ^{ab}	< 2.00 ^b	5.46 ^{abc}	3.70 ^c	4.76 ^{ab}	1.61 ^b
4	38.16 ^{ab}	8.35 ^a	0.071 ^{ab}	< 2.00 ^b	< 2.00 ^c	3.64 ^c	5.84 ^a	1.89 ^b
5	39.42 ^{ab}	9.79 ^a	0.070 ^{ab}	< 2.00 ^b	< 2.00 ^c	3.66 ^c	6.06 ^a	1.45 ^b
6	37.43 ^{ab}	9.67 ^a	0.066 ^b	< 2.00 ^b	3.07 ^{bc}	3.63 ^c	6.14 ^a	1.36 ^b
7	35.78 ^{ab}	8.86 ^a	0.078 ^{ab}	< 2.00 ^b	6.37 ^{ab}	3.86 ^b	4.02 ^b	3.90 ^a
8	40.20 ^a	7.99 ^{ab}	0.065 ^b	< 2.00 ^b	5.61 ^{abc}	3.78 ^{bc}	4.19 ^b	2.02 ^b
SEM	1.39	0.96	0.005	0.40	1.13	0.04	0.43	0.48
P value	0.03	0.001	0.04	0.001	0.001	0.001	0.001	0.001

¹Items measured were dry matter (DM, %), water soluble carbohydrates (WSC, % DM), ammonia-N (NH₃-N, % DM), yeasts (log₁₀ cfu/g), lactic acid bacteria (LAB, log₁₀ cfu/g), lactic acid (LA, % DM), and acetic acid (AA, % DM).

Table 3. Effect of plastic type on measurements following 12 mo of ensiling.

	DM	WSC	NH ₃ -N	LAB	pH	LA	AA	AS
Plastic type								
3	39.44 ^{bc}	4.86	0.112 ^{ab}	5.74 ^a	3.73 ^c	5.68 ^{ab}	2.20 ^b	158 ^{bc}
4	40.68 ^b	6.81	0.121 ^{ab}	5.42 ^a	3.72 ^c	5.89 ^{ab}	2.23 ^b	196 ^{bc}
5	39.97 ^b	6.05	0.131 ^{ab}	5.98 ^a	3.81 ^{bc}	4.69 ^{ab}	3.12 ^b	232 ^b
6	40.35 ^b	5.99	0.118 ^{ab}	3.10 ^b	3.75 ^{bc}	6.04 ^a	1.86 ^b	186 ^{bc}
7	36.23 ^c	3.51	0.144 ^a	6.75 ^a	4.11 ^a	1.93 ^c	5.60 ^a	94 ^c
8	44.96 ^a	6.18	0.104 ^b	6.01 ^a	3.91 ^b	3.96 ^{bc}	3.49 ^{ab}	>400 ^a
SEM	0.85	0.74	0.007	0.43	0.04	0.47	0.53	31
P value	0.001	0.06	0.01	0.001	0.001	0.001	0.001	0.001

Conclusions

In conclusion, the thin polyethylene and thin oxygen barrier plastics (numbers 1 and 2, respectively) did not withstand the 12-mo storage period, suggesting that although they had the same thickness as other thin plastics evaluated, their mechanical properties such as resistance to piercing might have been poorer than the other thin plastics. In addition, the standard polyethylene plastic (number 7) had the poorest silage fermentation and aerobic stability when compared to the oxygen barriers after long term storage which confirms the benefits of using oxygen barriers as silage covers.

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Homolactic Inoculant Effect on Bacterial Community of Triticale Harvested at Low and High Dry Matter

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Keywords: bacterial abundance, dry matter, lactic acid bacteria, triticale.

Introduction

Recommendations are that grass silages, like triticale, should be wilted and ensiled with DM contents of at least 30% to reduce the chance of clostridial fermentations. Triticale is often harvested in the spring when wilting conditions can be challenging because of cool ambient temperatures and frequent occurrences of rain. The variable spring weather results in crops ensiled over a wide range of DM, often being harvested too wet because of poor wilting conditions. Inoculants with homolactic acid-based microorganisms can increase the speed and improve the efficiency of silage fermentations dropping the pH quickly, which tends to discourage the growth of undesirable bacteria. The objective of this experiment was to evaluate the effects of a microbial inoculant containing classical homolactic acid bacteria on the bacterial community of triticale harvested at about boot stage of maturity and ensiled at a low (about 26%) and normal (about 34%) DM.

Materials and Methods

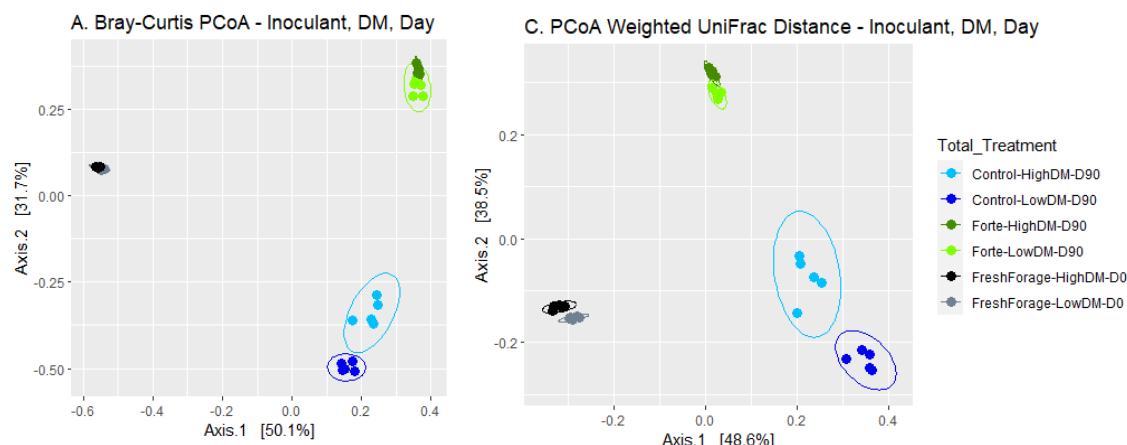
Triticale was harvested and either direct chopped at LOW DM (25.65% DM) or allowed to wilt until it was chopped at HIGH DM (33.88% DM). Forage at each DM level was split into 10 replicate piles (5 per treatment) that were treated with 1 of 2 additives, either water (CTRL) or 300,000 cfu/g of a mixture of *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Lactococcus lactis* (FRTE, Bonsilage Forte, Provita Supplements). The 2 × 2 factorial treatment structure resulted in 4 treatments: CTRL-LOW, FRTE-LOW, CTRL-HIGH, FRTE-HIGH. Approximately 800 g of forage from each pile was placed in a 25.4 × 35.6 cm nylon/polyethylene bag and vacuum sealed. Bags were stored at 22°C and opened following 90 d of ensiling. Microbial DNA was extracted from D0 fresh forage and D90 triticale silage cell pellets using the QIAamp PowerFecal Pro DNA Kit (QIAGEN, Hilden, Germany). Extracted DNA pellets were frozen and sent to RTLGenomics (Lubbock, TX, USA) for sequencing. The V4-V5 region of 16S rRNA gene was amplified using universal primers (515yF/926pF) and sequenced using normalized DNA pools and dual-barcoded Illumina MiSeq library preparation (RTL Genomics, Lubbock, TX, USA). Sample sequences were denoised and filtered using DADA2 within QIIME2. Taxonomic assignments were made against the SILVA_132_99_16S database. The resulting ASV tables were exported to R for normalization and statistical analysis and visualizations using the “phyloseq” package. Alpha diversity measures were tested with ANOVA and Tukey’s multiple pairwise tests if normally distributed or Kruskal-Wallis and Pairwise Wilcoxon Rank Sum test when non-normal. Statistical analysis of the beta diversity distance matrices was calculated with PERMANOVA using the ‘adonis’ and ‘betadisper’ functions in the ‘vegan’ R package. Pairwise PERMANOVA was calculated using the ‘pairwise.perm.manova’ function in the ‘RVAideMemoire’ R package. Taxa bar plots were created using the ‘microbiome’ and ‘microbiomeutilities’ R packages and averaging by treatment. Bacterial abundances were statistically compared using Wilcoxon Rank Sum Tests.

Results

Alpha diversity metric “observed species” measures the number of amplicon sequence variants (ASVs) present within each sample, describing sample richness. Shannon Index measures the observed ASVs within a community and the evenness of their abundance. For observed species, fresh forage had the highest median values, with CTRL and FRTE silages displaying approximately half the taxa present in the fresh samples (data not shown). When comparing CTRL-LOW to FRTE-LOW at d 90, there was no difference in either the observed species or Shannon index ($P > 0.10$ for both pairwise comparisons). However, there were differences between CTRL-HIGH and FRTE-HIGH ($P < 0.01$ for pairwise comparisons within both indices), with greater alpha diversity observed in FRTE compared to CTRL. Generally, less alpha diversity is favorable in silage, demonstrating dominance by lactic acid bacteria, suggesting that the FRTE treatment helped to drive fermentation for the HIGH DM.

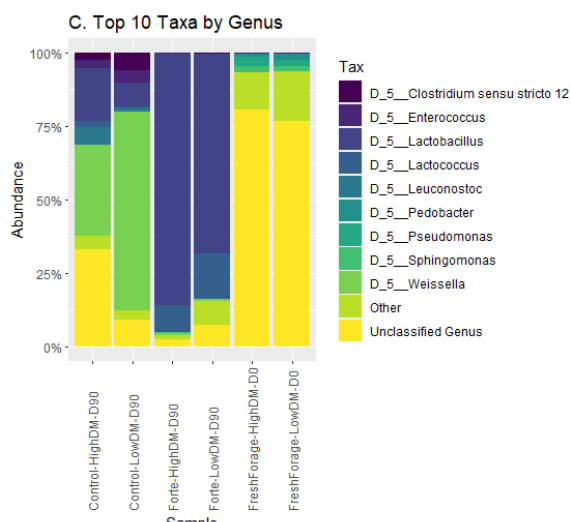
Beta diversity compares samples to each other, providing a measure of the distance or dissimilarity between each sample. Bray-Curtis Dissimilarity matrix measures community composition using an abundance-based approach to calculate dissimilarity between all samples, and the Weighted UniFrac method uses phylogenetic relatedness in addition to abundance counts. CTRL-LOW, CTRL-HIGH, FRTE-LOW, and FRTE-HIGH all differed from one another when evaluated using pairwise comparisons, but clustering is more strongly driven by the inoculant than the forage DM (Figure 1).

Figure 1. Beta diversity principal component analysis (PCoA plots) comparing treatments using Bray-Curtis Dissimilarity and Weighted UniFrac indices.



Taxa bar plots were generated at the genus level (Figure 2). The *Lactobacillus* and *Lactococcus* genera dominated the FRTE silages, comprising 68% and 15% of the LOW DM silage and 86% and 9% of the HIGH DM silage, respectively. The primary genera found in the CTRL silages were *Weissella*, unclassified *Enterobacteriaceae*, and *Lactobacillus*, comprising 68%, 9%, and 8% for LOW DM and 31%, 33%, and 18% for HIGH DM, respectively. In addition, undesirable organisms from the genera *Clostridium sensu stricto 12* and *Enterococcus* accounted for an average of 4.1% and 3.5% of observations for CTRL, respectively, but only <0.1% and 0.2% for FRTE, respectively.

Figure 2. Relative abundance taxa bar plots of the top 10 bacteria at the genus level.



Conclusions

The FRTE inoculant containing a mixture of *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Lactococcus lactis* altered bacterial communities of triticale harvested at LOW and HIGH DM. For FRTE silages, fermentation was likely dominated by organisms in the inoculant. In addition to lactic acid bacteria, the CTRL silages contained considerable representation by undesirable organisms such as *Enterobacteriaceae*, particularly for the high DM silage. The FRTE treatment was able to promote a favorable bacterial composition at both LOW and HIGH DM.

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Aerobic Deterioration of Diets Containing Cassava Shoot and Root Silages

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Keywords: aerobic stability, non-conventional feed, *Manihot esculenta*, silage

Introduction

The use of cassava and its derivatives in the form of silage as a non-conventional animal feed is common practice among farmers in the world. The appropriate fermentation profile for cassava aerial part and root silages, with pH and lactic acid values within the recommended ranges (Mota et al., 2011; Garcez et al., 2022) and considerably high aerobic stability (Santos et al., 2020; Pitirini et al., 2021) have been observed.

However, when silage is mixed with other feeds and fed to animals, its stability is altered (Jobim et al. 2007). We believe that the common evaluation of silage aerobic stability in a temperature-controlled ambient provides treatment comparison but does not show its actual behavior in the environment, nor possible interactions with the diet fed to the animal. It is therefore important to study the aerobic stability of diets containing silages. Therefore, the aim was to evaluate the aerobic stability of diets composed of cassava shoot and cassava root silages under field conditions.

Materials and methods

The experiment was conducted at the Farm School of the Federal Rural University of the Amazon – UFRA, located in the municipality of Igarapé-Açu, Pará, (01°07'21" S and 47°36'27" W). A completely randomized design was used, distributed in a 2×2 factorial, considering two sources of roughage (whole plant corn silage - WPCS; and cassava shoot silage - CSS) and two sources of energetic concentrate (ground corn - GC; and cassava root silage - CRS) in the composition of the diets, with four repetitions. The experimental diets were: WPCS+GC, WPCS+CRS, CSS+GC and CSS+CRS. The ingredients (roughage and concentrate) were manually mixed and the diets placed in plastic buckets (2 kg fresh matter) without compression. On days 0, 4 and 7 of the aerobic exposure period, the microbiological population was assessed using the Pour Plate technique, using specific culture media to identify Lactic Acid Bacteria (LAB), enterobacteria (Entero), yeasts and molds. The pH of the diets was measured on the same days. The temperature of diets and environment was recorded every 3 hours using digital thermometers, during seven days. Based on the temperature data, the following variables: aerobic stability (AS; days), time to reach maximum temperature (TMT, hours) and maximum temperature (MaxT, °C). Aerobic stability (AS) was the number of days it took for the silage to reach 5°C above ambient temperature.

Analysis of variance was carried out to test the effects of the roughage source (R), energetic concentrate (C), day of aerobic exposure (AE; 0, 4 and 7), and their interactions, for microbial and pH data. The parameters AS, TMT and MaxT were evaluated considering the effects of R, C and the interaction R×C. All analysis were performed using the SAS statistical software (SAS, 2022). The means were compared using the Tukey test, considering $\alpha=0.05$.

Results

There was an interaction between R×C×AE ($P < 0.05$) for LAB and yeast counts and for pH (Table 1). The LAB population did not differ among diets on days 0 and 7 of AE, but on day 4 it was higher in WPCS+CRS ($6.70 \log_{10} \text{ cfu.g}^{-1}$) and WPCS+GC ($5.38 \log_{10} \text{ cfu.g}^{-1}$) than the diets containing CSS, (Figure 1). Over the exposure period, there was a decrease ($P < 0.05$) in LAB from day 0 to day 4 in CSS+GC diet. For WPCS+GC and CSS+CRS, the LAB count did not differ over the AE period. For the enterobacteria population, there was no difference among diets, with counts below $1 \log_{10} \text{ cfu.g}^{-1}$ on day 0. However, the count of this microorganism was higher throughout the aerobic exposure period ($P < 0.05$). Diets containing CSS or CRS showed lower counts of enterobacteria compared to WPCS diets ($P < 0.05$). For yeasts, there was a difference between the diets only on day 0, being higher in WPCS+GC ($6.06 \log_{10} \text{ cfu.g}^{-1}$), but it was not different from CSS+CRS ($5.25 \log_{10} \text{ cfu.g}^{-1}$) (Figure 1). Diets CSS+GC and WPCS+CRS had the lowest counts (4.74 and $4.29 \log_{10} \text{ cfu.g}^{-1}$, respectively), but did not differ from CSS+CRS. The yeast population increased in all diets from day 4 onwards. As for pH on day 0, CSS+CRS (3.82) and WPCS+CRS (4.09), showed lower pH, but did not differ from CSS+GC (4.39). From day 4 onwards, all the diets had a pH above 7 (Figure 1).

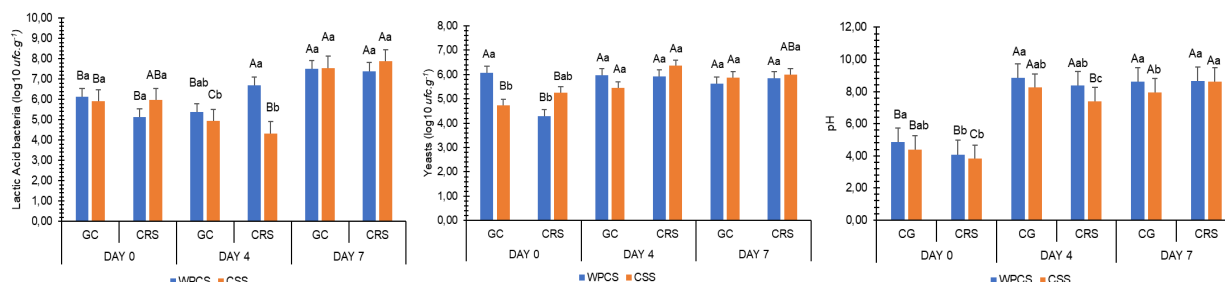
There was a R×C interaction for AS ($P < 0.01$), TTmax ($P < 0.01$) and Tmax ($P < 0.01$) (Table 1). WPCS+GC had the lowest AS (0.44 days; $P < 0.05$). CSS+GC and CSS+CRS did not differ ($P > 0.05$), with stability of 5.41 and 4.75 days, respectively, followed by WPCS+CRS, with stability of 3.38 days. These diets had higher ($P < 0.05$) TTmax and lower ($P < 0.05$) Tmax.

Table 1. Microbiology ($\log_{10} \text{ cfu.g}^{-1}$), pH, aerobic stability (AS), time to maximum temperature (TMT) and maximum temperature (MT) of diets composed of whole plant corn silage (WPCS), cassava shoot silage (CSS) and cassava root silage (CRS) throughout the aerobic exposure period.

LAB: acid	<i>Itens</i>	WPCS		CSS		AE			SEM	R	C	R×C	R×C×DAE	lactic
		GC	CRS	GC	CRS	0	4	7						
		<i>P value</i> ¹												
	LAB, log10 <i>cfu</i> ⁻¹	6.33	6.41	6.13	6.06	5.78	5.34	7.58	0.26	0.24	0.97	0.75	0.03	
	Entero, log10 <i>cfu</i> ⁻¹	4.47	3.54	4.76	4.36	0.22	5.26	7.24	0.79	<0.01	0.04	0.48	0.08	
	Yeast, log10 <i>cfu</i> ⁻¹	5.87	5.48	5.35	5.87	5.16	5.93	5.83	0.10	0.94	0.91	<0.01	<0.01	
	Mold, log10 <i>cfu</i> ⁻¹	7.29	6.27	5.72	5.49	5.31	6.22	6.91	0.23	<0.01	<0.01	<0.01	0.07	
	pH	7.43	7.04	6.86	6.65	4.28	8.28	8.46	0.52	<0.01	<0.01	0.21	0.04	
	AS, <i>days</i>	0.44	3.38	5.41	4.75	-	-	-	0.69	<0.01	<0.01	<0.01	-	
	TMT, <i>days</i>	3.13	5.63	6.88	6.72	-	-	-	0.55	<0.01	<0.01	<0.01	-	
	MT, °C	48.84	46.83	43.95	46.00	-	-	-	0.56	<0.01	0.96	<0.01	-	

bacteria; Entero: enterobacteria; DAE: day of aerobic exposure; SEM: standard error of the mean; R: effect of roughage; C: effect of concentrate; R×C: interaction between roughage and concentrate; (R×C)×DAE: interaction between roughage, concentrate and day of aerobic exposure. ¹ $\alpha = 0.05$.

Figure 1: Averages ($\log_{10} \text{ cfu.g}^{-1}$) of lactic acid bacteria (LAB), yeasts and mold, and pH in diets composed of whole plant corn silage (WPCS), cassava shoot silage (CSS) and cassava root silage (CRS) throughout aerobic exposure.



GC: ground corn. Averages followed by different lowercase letters differ from each other during the same aerobic exposure period and averages followed by different uppercase letters differ from each other throughout the aerobic exposure period, according to the Tukey test ($P < 0.05$).

Conclusions

Diets containing CSS and CRS provided greater resistance to aerobic deterioration compared to diets containing of WPCS and GC, showing a longer shelf life under field conditions.

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Cost Comparison Between Corn and Cassava Peel Silage In Diets for Feedlot Beef Cattle

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Keywords: energy, nutrition, ruminants.

Introduction

The flour industries use about 80% of cassava, making its derivatives available in large quantities. The main residue is cassava peel, obtained by peeling the roots and consisting of peel, inner bark, cortex debris and root tips. Cassava peel is suitable for silage production, has low hygroscopic power, that is, it has difficulty retaining water, being a perishable food if not preserved properly (Prado, 2006; Faria et al., 2011; Vilhalva et al., 2012). However, studies demonstrate that cassava peel silage has the potential to be used as an energy source in the diet of ruminants (Lounglawan et al., 2011). The objective was to assess the impact of price on the feasibility of using cassava peel silage in feedlot systems under different economic scenarios.

Materials and methods

Samples of the diet components (ground corn (*Zea mays*), cassava peel silage (*Manihot esculenta*), guinea grass silage (*Megathyrus maximus* cv. Mombaça), soybean meal (*Glycine max*) were obtained from rural establishments in the northeast region of Para state. Analyses of dry matter (DM), mineral matter (MM), crude protein (CP), neutral detergent fiber (NDF) and non-fibrous carbohydrates (NFC) were performed according to the methodology described by Detmann et al., (2021). The total digestible nutrients were also calculated according to Weiss' equation, described by Medeiros et al., (2015). The chemical composition of food can be seen in Table 1.

Table 1 - Chemical composition of the ingredients

Ingredients	DM	OM	CP	EE	NDF	NFC	TDN
Ground corn	86,90	98,69	8,69	5,54	13,06	71,39	88,23
Cassava peel silage	33,57	97,32	2,36	0,60	12,81	81,55	76,42
Mombaça silage	33,09	92,33	5,14	1,43	74,25	11,51	48,91
Soybean meal	89,02	92,52	45,51	1,00	28,84	17,17	69,48

DM: Dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; NFC: non—fibrous carbohydrates; TDN: total digestible nutrients.

After the result of the chemical composition, a simulation was carried out with three scenarios (varying ingredients and costs), by simulating a feedlot diet for male Nelore cattle (500 kg of live weight) with an average daily gain of 1.3 kg.day. The roughage:concentrate ratio was 25:75 and the roughage source was guinea grass silage. The scenarios were: Scenario 1 (GC): reference diet containing ground corn; Scenario 2 (CPS-LowPrice): diet containing cassava peel silage purchased with a low price; Scenario 3 (CPS-HighPrice): diet containing cassava peel silage purchased with a high price. The purchase prices of the dietary ingredients were: CPS low-cost (0.74 BRL/kg DM; 0.13 USD/kg DM); CPS high-cost (1.49 BRL/kg DM; 0.26 USD/kg DM). ground corn (2.07 BRL/kg DM; 0.36 USD/kg DM) soybean meal (3.60 BRL/kg DM; 0.62 USD/kg) and guinea grass silage (0.91 BRL/kg DM; 0.16 USD/kg DM). The exchange rate from Brazilian Real (BRL) to US Dollar (USD) was 5.84, and the sale prices of the ingredients used were found in the region of northeast of Pará state. The nutritional requirements of DM, CP and TDN are shown in the Table 2.

Table 1 – Average daily gain (ADG) and nutritional requirements for dry matter (DM), crude protein (CP) and TDN intake of a male Nelore cattle with 500 kg live weight, according to BR-Corte

ADG (kg/day)		DM (kg/day)	CP (kg/day)	TDN (kg/day)	CP (%)	TDN (%)
1,3	Requirements	10,21	1,08	6,85	10,59	67,09

Results

The results presented in Table 3 shows that the inclusion of CPS in the diet led to increase in the soybean meal inclusion, compared with the diet of Scenario 1. The inclusion of CPS in the diets decreased the dietary TDN compared with the reference diet of Scenario 1. The results also showed that the replacement of ground corn by CPS in the diets can meet the nutritional requirements of feedlot cattle.

Although Scenario 2 requires a higher quantity of soybean meal to meet the nutritional demands, it still presents a lower cost than Scenario 1. The inclusion of CPS in the Scenario 2 represents a reduction of 26.5% in the diet cost. However, when the CPS had a high price (Scenario 3), the reduction diet cost was only 6.9%. The daily cost, which is related to the DM intake, was also 26.5% lower in the Scenario 2 compared with Scenario 1.

Table 2 - centesimal composition of foods, chemical composition and the kg/DM cost of diets.

	Scenario 1 GC	Scenario 2 CPS – Low price	Scenario 3 CPS – High price
Centesimal composition (%DM)			
Guinea grass silage	25	25	25
Ground corn	63	-	-
Cassava peel silage	-	52	52
Soybean meal	12	23	23
Chemical composition (%)			
Crude protein	12.22	12.98	12.98
Total digestible nutrients	76.15	67.17	67.17
Neutral detergent fiber	30.25	31.86	31.86
Feeding Costs			
Diet cost (BRL/kg DM)	1.96	1.44	1.83
Diet cost (USD/kg DM)	0.34	0.25	0.31
Daily dietary costBRL/animal.day)	20.01	14.70	18.86
Daily dietary costBRL/animal.day)	3.47	2.55	3.17

GC= ground corn; CPS= cassava peel silage

In Scenario 3, where cassava peel silage costs are slightly lower than Scenario 1. However, the costs associated with the logistics of storing cassava peel silage should be considered to prevent losses (Faria et al., 2011). The lack of standardization of the nutritional composition of these derivatives must be considered, as it can vary according to processing, climatic and seasonal conditions (Woiciechowski et al., 2013).

Conclusions

The nutritional analysis shows that cassava peel silage can meet the nutritional requirements of feedlot cattle. However, its use is suitable when the prices are low (0.74 BRL/kg DM; 0.13 USD/kg DM).

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Productive Performance of Feedlot Sheep Fed Cassava Peel Silage with or Without Palm Kernel Cake Replacing Ground Corn

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Keywords: wheight gain, sustainability, tropical silages

Introduction

Brazil has a wide diversity of foods destined for agro-industries, among which cassava and palm oil stand out, being the fourth largest producer of cassava (CONAB, 2023) and the majority of palm oil production is concentrated in the North and Northeast regions of the country (COSTA et al., 2011). As a result of agro-industrial processing, by-products of these foods are generated and can be used to feed ruminants, which play an important role in the bioeconomy, in which they convert foods that are inedible for humans (forage, agricultural by-products, crop residues) into high-value food (meat and milk) (OLIVEIRA et al., 2014). Cassava peel and palm kernel cake are unconventional foods from the agroindustry that present viable nutritional values for feeding ruminants, being used as a source of energy in animal feed (SANTOS et al., 2019). However, cassava peel may contain certain levels of hydrogen cyanide (HCN), which are compounds that are harmful to animals. Strategies such as fermentation are used to reduce these levels (PITIRINI, 2023).

The goal of this experiment was to evaluate the performance of feedlot sheep fed diets with cassava peel silage and cassava peel silage with palm kernel cake replacing the energy in the diet concentrate.

Materials and methods

The experiment was conducted in the experimental area at the Igarapé-Açu School Farm of the Federal Rural University of the Amazon (UFRA), located in the municipality of Igarapé-Açu, in the state of Pará, Brazil, 01°07'21" S e 47°36'27" W. Thirty crossbred sheep, male and female, with a predominance of Santa Inês breed, healthy and dewormed (Albendazol 10% - Oral solution; 5 mg/kg live weight), with an average initial weight of 18 kg were used and distributed through a randomized block design, using the sex of the animals as a blocking factor, with two blocks and three treatments and five repetitions, totaling thirty experimental units. The experimental diets were formulated to be isoproteic, meeting the nutrient requirements for lambs with an average daily weight gain of 200g, according to Nutrients Requirements of Small ruminant's recommendations (2007). In this way, the diets were the replacement of the source of energy ingredient in the concentrate. Diet 1 was composed of guinea grass silage, ground corn and soybean meal. Diet 2 and diet 3 were composed of cassava peel silage and cassava peel silage + 30% of palm kernel cake, respectively, replacing ground corn. The chemical analysis of the ingredients was carried out in the animal nutrition laboratory of the Federal Rural University of the Amazon.

The feedlot evaluation lasted 97 days, with 20 days for adaptation and 67 days for collecting data for intake, feed conversion and weight gain. All variables were analyzed using the GLM procedure in the Statistical Analysis System (SAS). The means were compared by the Tukey test, considering $\alpha = 0.05$.

Table 1 - Chemical composition of ingredients and experimental diets

CPS= cassava peel silage; PKC = palm kernel cake

Results

Items	Dry matter (%)	Organic matter	Crude protein	Ether extract	NDF	ADF	non-fibrous carbohydrates	Initial live weight and DM intake
Guinea grass silage	37.88	93.27	3.48	1.13	76.12	47.56	12.54	
Ground corn	92.57	98.62	7.26	4.89	17.29	3.95	69.18	
CPS	38.65	94.76	4.68	1.28	20.74	14.11	68.06	
CPS+ PKC	51.09	83.52	5.10	4.38	49.49	31.10	24.55	
Soybean meal	93.56	93.39	41.71	7.52	22.06	13.02	22.10	
Diet 1	69.65	93.41	15.71	7.01	42.88	21.04	32.74	
Diet 2	53.94	92.44	15.08	5.39	31.90	22.30	43.91	
Diet 3	60.06	87.03	15.69	8.20	48.05	27.99	20.32	

expressed as % LW were not affected by the diets ($P>0.05$; Table 2).

The performance parameters, DM intake (kg/day) and the feed efficiency were affected by the diets ($P<0.05$) in the same way. Diets containing CPS showed similar values of the diet containing GC, and both CPS and GC showed higher values compared with the diet containing CPS+PKC.

Table 2 - Average values of initial weight, final weight, total weight gain, daily weight gain, dry matter intake and feed efficiency of feedlot sheep.

Items	Initial live weight	Final live weight	Total weight gain	Daily weight gain	Dry matter intake		Feed efficiency
	kg	kg	kg	kg/day	kg/day	%LW	kg gain/KgDM
Ground corn	19.11a	31.09a	11.98a	0.179a	0.901a	3.59a	0.201a
CPS	18.40a	30.34a	11.94a	0.178a	0.911a	3.75a	0.196a
CPS+PKC	18.50a	25.27b	6.77b	0.101b	0.765b	3.53a	0.133b
SE	0.53	0.73	0.54	0.00	0.02	0.05	0.00
P-value							
Diet	0.67	<0.01	<0.01	<0.01	<0.01	0.23	<0.01

CPS= cassava peel silage; PKC = palm kernel cake

The low intake and performance of CPS+PKC diet relies on its high NDF concentration due to the inclusion of palm cake in cassava peel silage, which increases the dry matter and fiber content in the silage (PITIRINI, 2023). A diet with high NDF can decrease DM intake. The high proportion of fiber influences consumption, due to the peculiar characteristics of the digestive tract of ruminants, which include long periods of food retention and a large storage capacity in the rumen (VISONA-OLIVEIRA et al., 2015). In addition, other factors can affect silage intake, as the product quality and animal's preference.

The results show that cassava peel silage can replace ground corn in concentrate diets for ruminants without altering the productive performance of the animals. In their study, supplementing sheep with fresh, dried and ensiled cassava peel, Niayale et al (2020) shows that the use of cassava peel silage provides better results in animal performance. Cassava peel has a high potential for total replacement of corn as it has similar energy values (SANTOS et al., 2015).

Conclusions

Cassava peel silage can be used to replace ground corn concentrate in animal diets for sheep as it does not alter the animal's productive performance characteristics and can optimize the production costs of small ruminants. The addition of palm kernel cake to cassava peel silage did not show effective results in the productive performance of sheep, as high fiber values in diets can influence the animal's feed intake.

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Obligate Heterofermentative Lactic Acid Bacteria Decrease Losses During Storage and Feedlot in Corn Silage Fermented for Long Period

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Keywords: aerobic stability, inoculant, lactic acid bacteria, yeast

Introduction

Corn silage is a major source of forage for lactating dairy cattle worldwide. However, corn silage can spoil rapidly when exposed to air during storage or feedout. The development of spoilage microorganisms result in losses of nutrients, animal performance and health issues. Silage inoculants containing obligate heterofermentative lactic acid bacteria (LAB) has been proven capable of improving aerobic stability of silage by increasing acetic acid formation, with a slightly increase of DM loss during fermentation (0.78%-unit in corn silage; Arriola et al., 2021). Nowadays, several farms are storing corn silage for prolonged periods, not only as a strategy to improve starch digestibility, but also to decrease the risk of forage shortage. Although acetic acid concentration tends to increase with prolonged storage (Kung, 2013), air infiltration into the silo likewise increases with storage time, increasing the risk of aerobic deterioration. The objective of this study was to examine the effect of a silage inoculant based on heterofermentative LAB on the fermentation and aerobic stability of corn silage stored for approximately 1.5 year.

Materials and methods

Whole-plant corn (hybrid JM 2M91PRO3) was harvested at 3/4 kernel milk line (~42% dry matter (DM)) from two field plots. Chopped forage (12-mm theoretical length of cut) from each plot was divided into 8 piles (6 kg per pile). From each plot, four piles were inoculated with 1.5×10^5 CFU g⁻¹ of *Lentilactobacillus buchneri* NCIMB 40788 plus 1.5×10^5 CFU g⁻¹ of *Lentilactobacillus hilgardii* CNCM I-4785 (Magniva Platinum 1, Lallemand; **LBH**), and four piles were assigned as control (**CON**) before ensiling in 7.2-L plastic buckets. The inoculant was diluted in distilled water and applied at 10 mL kg⁻¹. The control received the same volume of water. The combination of two field plots, two treatments and four replicates resulted in a total of 16 silos. The experimental silos were stored in a closed barn at room temperature (15-32 °C). After 520 d of storage, silages were sampled to measure DM, microbial counts, and fermentation products by wet chemistry methods. Silage DM content was corrected for loss of volatile compounds during drying (Weissbach and Strubelt, 2008). After feedout, silage aerobic stability was defined as the time elapsed until silage temperature reached 2 °C above room temperature (25 ± 1.8 °C), during 10 d of air exposure. Dry matter loss was measured both during 520 d of storage and during 10 d of aeration after feedout. Data were analyzed using the proc MIXED of SAS including a random effect of plot and fixed effect of treatment.

Results

Inoculation with LBH resulted in higher concentrations of lactic acid, acetic acid, and 1,2-propanediol, whereas decreased ethanol formation and pH compared to CON. The NH₃-N concentration was not affected by treatment. Higher counts of LAB and lower counts of yeasts and molds were observed in corn silage inoculated with LBH. Inoculated silage also had higher DM concentration than the CON silage. The application of LBH reduced DM loss during storage from 7.46 to 5.98%, as the risk of air infiltration might have increased by prolonged storage (i.e., 520 d). Furthermore, compared to CON, silage treated with LBH had longer aerobic stability and lower DM loss during 10-d aerobic exposure (11.8 vs 2.25%). Each day elapsed until silage temperature rise represented a reduction of 1.55%-unit in DM loss during feedout (Figure 1).

Conclusions

Inoculation of corn silage with obligate heterofermentative LAB was effective in inhibiting undesirable microorganisms and reduced DM losses both during a long-term storage (520 d) and feedout phases. Reducing DM loss caused by aerobic deterioration during storage offset the amount of CO₂ produced by heterofermentative LAB metabolism.

Table 1. Fermentation and aerobic stability of corn silage treated with heterolactic LAB inoculant and stored for 520 d

Item	Treatment ¹		SEM ²	P-value
	CON	LBH		
Dry matter, %FM	38.2	39.0	0.679	0.001
Lactic acid bacteria, log CFU g ⁻¹ FM	6.03	7.15	0.149	<0.001
Yeasts, log CFU g ⁻¹ FM	3.73	2.71	0.211	<0.001
Molds, log CFU g ⁻¹ FM	3.78	2.95	0.236	<0.001
pH	3.67	3.57	0.046	<0.001
NH ₃ -N, %N	12.2	12.1	0.328	0.808
Lactic acid, %DM	4.87	5.36	0.365	0.001
Acetic acid, %DM	1.50	2.99	0.209	<0.001
Ethanol, %DM	1.76	0.697	0.072	<0.001
1,2-Propanediol, %DM	0.003	0.680	0.110	<0.001
DM loss during 520-d storage, %DM	7.46	5.98	0.309	0.003
DM loss during 10-d aeration, %DM	11.8	2.25	0.475	<0.001
Aerobic stability, d	2.16	8.22	0.255	<0.001

¹ CON: Without inoculant; LBH: inoculation with 1.5×10^5 CFU/g of *Lentilactobacillus buchneri* + 1.5×10^5 CFU/g of *Lentilactobacillus hilgardii*. ² Standard error of the mean.

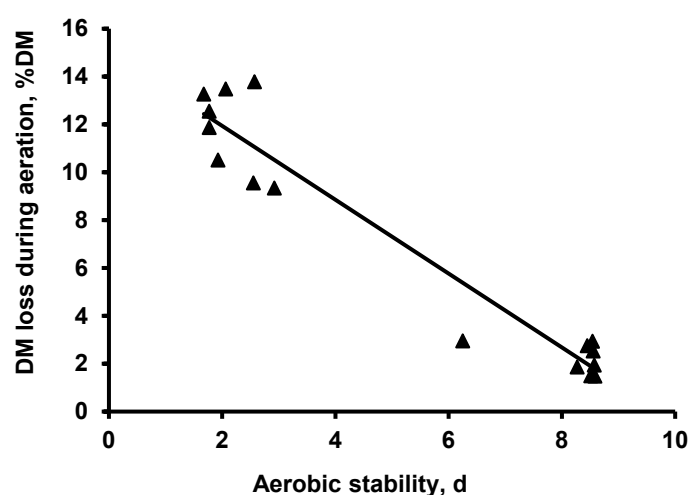


Figure 1. Relationship between aerobic stability (AS) and DM loss during 10-d aeration. DM loss during aeration (%DM) = $15.1 - 1.55 \times AS$ (d); RMSE = 1.35; R² = 0.935; P < 0.001.

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Optimizing Silage Production in Tropical Climates

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Keywords: feeding value, forage, grain, nutrient recovery

Introduction

In the last half century, technological advances in tropical areas have increased food and fuel production to face malnutrition, environmental degradation, and climate change. Nowadays, tropical agriculture contributes significantly to global trading, including the supply of animal protein for internal and external markets. In this context, silage is being produced in tropical areas not only to supplement cattle during pasture shortage in grazing systems but also for feeding housed dairy cows and beef feedlots all year round. For instance, in Brazil, the number of cattle finished in feedlots increased from 3.8 million in 2018 to 7.2 million of heads in 2023 (i.e., ~21% of the slaughtered cattle; Cepea/CNA), while milk production from housed cows increased from 16% in 2019 to 44% of the total ~35.4 billion liters in 2022 (MilkPoint Ventures/IBGE). These trends indicate an intensification of beef and dairy production, which aligns with a greater demand of high-quality silage. Furthermore, we are now seeing an increased demand for silage consultants in the market. In the meantime, silage systems are still heterogeneous, with many smallholders, as in other tropical regions. This paper provides an overview of recent advances and opportunities for silage production in tropical climates, bringing the Brazilian experience as a case.

Recent advances and opportunities for silage production in tropical climates

Whole-plant corn silage is the major primary forage source in Brazilian diets for both beef feedlots (~56% of respondents; Monsalve and Millen, unpublished) and housed dairy cows (100% of respondents; Bernardes et al., 2018), with low-starch forages been used as secondary roughage sources (e.g., tropical and temperate grasses, sorghum, sugarcane, pearl millet, winter cereals, alfalfa). However, the increased agronomical challenges observed in the last growing seasons have stimulated the adoption of practices to improve corn plant health, such as choosing corn hybrids with greater tolerance to pests and diseases (e.g., corn stunting pathogens transmitted by leafhopper), improving plant nutrition, and applying fungicides. At harvest, corn silage processing targeting both kernel breakage and appropriate forage particle size has improved over the last decades, likely due to the wider availability of self-propelled forage harvesters and the adoption of on-farm tools to monitoring corn plant processing at harvesting. Nowadays, there is a claim that approximately half of the Brazilian corn silage tonnage is harvested by self-propelled forage harvesters (it was ~10% ten years ago). Recently, Salvati et al. (2021) showed that dairy cows fed flint corn silage harvested with a self-propelled harvester with processing rollers produced 1.2 kg/d more milk than cows fed silage harvested with a pull-type machine without a kernel processor. Moreover, Piran Filho et al. (2023) demonstrated that feeding dairy cows with corn-silage particles >19 mm promoted sorting against long particles and diet NDF, decreased DM intake, milk (-2 kg/d) and component yields, compared with cows fed corn-silage particles with 8 to 19 mm. Hence, there is still room for improving corn silage harvest practices as farmers become aware of those benefits.

Beyond optimal silo filling and packing, proper sealing and feedout, and application of heterofermentative lactic acid bacteria (LAB) based inoculants have been proved to curtail aerobic deterioration and improve corn silage feeding value in tropical areas. Parra et al. (2021) reported that replacing a standard sealing method based on a polyethylene film with an oxygen barrier sealing system based on an ethylene-vinyl alcohol film protected with a woven anti-UV cover improved corn silage conservation, aerobic stability of TMR, DM intake, and performance of growing dairy heifers (+130 g/d). Bernardes et al. (2021) demonstrated that a daily removal rate of over 250 kg of silage/m² markedly reduced the risk of spoilage in corn silage, as such index combines (indirectly) silage density and linear feedout rate in a single recording. Currently, different Brazilian research groups are selecting new LAB strains with potential as silage inoculant for crops cultivated in tropical areas (Carvalho et al., 2021).

While the production of corn silage in dairy farms seems unarguable; in beef feedlots, the frequency of corn silage reported by surveyed nutritionists decreased from 69% in 2019 to 56% in 2024, while tropical grass silage increased from 8.3 to 24% in the same period (Monsalve and Millen, unpublished). Also, in rations for growing cattle, the use of tropical grass silage enables nutritionists to formulate diets that balances energy intake, thereby avoiding that cattle gain weight too quickly, which would negatively impact carcass weight as animals reach their mature size. Furthermore, the increased risk of drought in recent growing seasons brought an additional motivation for planting crops less susceptible to drought, pests and diseases, as complimentary forages to corn silage. Yet, as tropical grasses (mainly *Megathyrsus* and *Urochloa* genera) are widely used in crop rotation systems within no-tillage practices, many sustainable farms are now producing tropical grass silages in integrated crop-livestock systems. However, tropical grasses are considered difficult to ensile, due to their low contents of DM (<28%) and soluble carbohydrates (SC; <4% of DM), while exhibiting a relatively high buffering capacity (BC; >4 g of lactic acid/100 g DM) at harvesting. In fact, in a recent survey in 51 Brazilian beef farms we observed that 86% of tropical grass silages had butyric fermentation, as most grasses were ensiled upon direct cut with a high moisture content (Bragatto et al., unpublished). At the same time, Carvalho et al. (2024) demonstrated that the fermentability coefficient (FC) concept (developed in Germany for temperate forages) also serves as a practical tool to predict the fermentation course in tropical grass silages. Additionally, they demonstrated based on an independent dataset (n = 222) that FC values ≥35 markedly decreases the risk of butyric fermentation, whereas FC values ≥40 completely suppress butyric fermentation

in tropical grasses with moderate levels of nitrate and LAB counts. The low SC:BC ratio frequently found in tropical grasses (~0.5) strongly suggested that the practice of wilting should be considered as tool to obtain forages with a DM content enough to induce good fermentation (i.e., >35%). When wilting is not feasible (due to machinery scarcity or weather conditions), chemical additives have been proven successful to curtail *Clostridium* development in silage. Recently, Gomes et al. (2021) demonstrated that chemical additives based on sodium nitrite inhibited clostridial fermentation and decreased nutrient losses in direct-cut tropical grass silage.

In addition to forage silages, the production of grain silages, including high-moisture corn, reconstituted corn, reconstituted sorghum, and snaplage, has increased as a strategy to improve starch digestibility, since most tropical corn and sorghum kernels have a high proportion of vitreous endosperm. Jacovaci et al. (2021) reported in a meta-analysis that the ensiling process improved the feeding value of flint corn grain by 25.7% for beef cattle. Therefore, the use of grain silages in Brazilian feedlots steeply increased from 3% to >36% (proportion of respondents) in the last decade (Monsalve and Millen, unpublished).

Because of silage expansion in tropical areas, most cattle diets contain a large proportion of fermented feeds. In some instances, the complete diet is ensiled (i.e., TMR silage), often for sale to smallholders or medium farmers expanding the herd in a limited plowing area. Several benefits have been associated with TMR silages, such as a reduced requirement for labor and machinery, uniform composition, potential for incorporating unpalatable feedstuffs, and high aerobic stability. Ensiling TMR has been also a sustainable alternative to efficiently handle wet byproducts in ruminant diets (Bueno et al., 2020). In drylands, spineless cactus has been used as an important ingredient in TMR or PMR silages supplied to smallholders (Santos et al., 2020).

Final remarks

Development and adoption of new technologies are innovating tropical agriculture, allowing a sustainable production of high-quality silages. Policies capable of transferring new knowledge to farmers all around the world might reduce silage quality variation, improve animal production efficiency and sustainability, and still expand silage with its central role in food production.

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Performance of Dairy Heifers Fed Whole-Plant Corn Silage Treated with Benzohydrazide

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Keywords: digestibility, enzyme inhibitors, feed intake, lignin

Introduction

Whole-plant corn silage has been the main source of forage in ruminant diets in intensive operations or during part of the year in pasture systems. Corn silage digestibility is mainly determined by starch and fiber contents and their respective digestibility. Fiber digestibility is mainly affected by lignification during plant development (Van Soest, 1994). A potential strategy to reduce the negative impact of lignin on the digestibility of forage fiber is the application of enzyme inhibitors of the phenylpropanoid pathway. For instance, benzohydrazide can regulate the biosynthesis of lignin monomers and thus modify the structure of this polymer present in the cell wall of corn plants (Martarello et al., 2023a), which may facilitate the attack of microbial hydrolases in the rumen. Thus, the objective of this study was to evaluate the performance of dairy heifers fed diets containing whole-plant corn silage treated with benzohydrazide.

Materials and methods

Corn hybrid DKB255PRO3 was sown in four 1,0-ha plots placed in two field blocks (two plots per block). After 26 days of plant emergence, one plot of each block was sprayed with 180 L ha⁻¹ of a solution 50 µM of benzohydrazide (**BZD**). At 43 days of plant emergency, BZD was reapplied to the same plots. The other two plots were untreated (**CON**). Whole-plant corn was mechanically harvested at approximately 35% dry matter (DM) and stored separately in two bunker silos (i.e., one silo for BZD and one silo for CON). After two months of storage, the silages were fed to 24 Holstein heifers (12 heifers per treatment) during 67 days (7 days of adaptation and 60 days of treatment comparison). Heifers were blocked by body weight (12 blocks) and housed in individual pens. Experimental diets contained 15% concentrate and 85% corn silage (CON or BZD). Individual dry matter intake (DMI) was recorded daily. At the beginning and end of the comparison period, animals were weighed, and the daily weight gain was computed by the difference between the final and initial body weights, divided by days of feed. Blood samples were collected by puncture of the jugular vein 4 h after the morning feed to determine glucose and urea concentrations. Total-tract apparent digestibility was estimated using indigestible NDF (iNDF) as an internal marker. Samples of TMR, leftovers and feces were collected daily for 5 consecutive days in the 4th and 7th weeks of comparison. Data were compared using the MIXED procedure of SAS with a model including a random effect of block and a fixed effect of treatment (CON or BZD). Significant differences were declared if $P \leq 0.05$, and trends if $P > 0.05$ and $P \leq 0.10$.

Results

Heifers receiving BZD had lower dry matter intake compared to CON. However, total-tract digestibility of DM and NDF were higher for BZD. Then, average daily weight gain was similar between treatments. Benzohydrazide is a molecule capable of regulating the biosynthesis of lignin monomers, such as p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S). In a previous study, when BZD was applied to growing corn plants, it enhanced biomass saccharification as revealed by enzymatic assay (Martarello et al., 2023b). In our study, BZD application during early stages of corn plant development might have altered the structure of lignin in the plant cell wall, as total lignin content was similar between treatments (not shown). Such alteration likely facilitated the attack of microbial hydrolases in the rumen. Consequently, corn silage treated with BZD increased feed efficiency by 11%, compared to CON. Plasma glucose concentration was not altered by treatments, but urea concentration was reduced in plasma of heifers fed BZD-treated corn silage, suggesting a greater availability of substrates for ruminal fermentation.

Table 1. Performance, apparent digestibility, and blood parameters of dairy heifers fed corn silage

Item	Treatment ¹		SEM ²	P-value
	CON	BZD		
Dry matter intake, kg d ⁻¹	11.0	10.2	0.23	0.039
Initial body weight, kg	334	333	7,64	0,944
Final body weight, kg	397	401	8,62	0,800
Daily weight gain, kg d ⁻¹	1.32	1.38	0.042	0.343
Feed efficiency	0.128	0.142	0.003	0.011
Total-tract DM digestibility, %	58.4	61.9	0.59	<0.001
Total-tract NDF digestibility, %	34.0	37.5	1.43	0.002
Glucose, mg dL ⁻¹	67.3	69.7	3.61	0.283
Urea, mg dL ⁻¹	25.1	22.7	1.29	0.009

¹ CON: silage control; BZD: silage treated with benzohydrazide.

²Standard error of the mean.

Conclusions

Application of benzohydrazide to corn crop increased fiber and dry matter digestibility *in vivo*, which increased the corn silage feeding value for growing dairy heifers.

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Optimizing Preference, Intake, and Performance of Silage Fed Ruminants

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Keywords: preference, silage intake, performance

Introduction

Silage is a major component of ruminant diets, providing essential nutrients and energy to the animals. Therefore, it is critical that we understand factors that affect silage quality. Chemical, microbial, and organoleptic characterization of silage has been used to explain the type of fermentation that occurred and give some indication of palatability and the likely animal performance (Kung et al., 2018). Good quality silage should be free of undesirable compounds that could negatively affect an animal's intake and ultimately its performance.

Volatile Organic Acids

Silage fermentation pathways and chemical and biochemical reactions occurring during the silage phases (i.e., aerobic, fermentation, and feed-out) have been extensively researched. There is formation of nonvolatile organic acids (e.g., lactic acid) and volatile organic acids (VOC; e.g., acetic acid, propionic acid, butyric acid, ethanol, and esters). Recently, Bandla et al., (2024) reviewed the impact of VOC on ruminant silage preference and dry matter intake (DMI). Interestingly, VOC influences silage preference and DMI through palatability and physiological effects including osmolality changes, rumen motility, hepatic oxidation and post-ingestive feedback. It has been suggested for many years that acetic acid and propionic acid to some extent negatively affect intake in ruminants. However, the results are equivocal (Grant and Ferraretto, 2018; Bandla et al., 2024). The specific mechanism or compounds affecting intake or preference have not been completely elucidated (Grant and Ferraretto, 2018). There is research suggesting that when heterofermentative bacteria increase acetic acid in silage (e.g., use of an inoculant to improve feed-out stability), that the effect of acetic acid on DMI is reduced or nonexistent (Bandla et al., 2024). We (Dann et al., 2025) evaluated the lactation performance of Holstein cows fed diets containing corn silage treated with a multi-strain inoculant (MSI) or not (CON) at harvest. The inoculant included *Lentilactobacillus buchneri*, *Lentilactobacillus hilgardii*, and *Pediococcus pentosaceus*. The MSI-treated silage had a higher acetate concentration and more good quality material available for feeding compared to the CON silage. Lactation performance and feed efficiency were not affected by treatment since only good quality silage was fed. Energy-corrected milk yield per material ensiled (kg/kg) was great for the MSI-treated silage when fermentation and spoilage losses were considered and a balanced diet was fed. This study confirmed the importance of using a well-defined and proven inoculant at harvest on chopped whole plant corn to maintain quality during ensiling and feed-out.

Palatability and Preference

Preference studies might provide better insight than infusion and silage intake models for understanding the effect of VOC on DMI (Bandla et al., 2024). An animal's feeding behavior and DMI of silage is a good estimate of a silage's palatability. The DMI is a good proxy for determining preference when an animal is offered 2 or more feeds under controlled conditions (Pape et al., 2024). Multi-dimensional scaling and Plackett-Luce model methodologies have been used in preference research with each having some limitations. Our group (Pape et al., 2024) proposed an alternative analysis approach that combined the multiverse analysis and the Bradley-Terry model methods, focused on use of behavioral data (i.e., dry matter intake) rather than chemical, microbial, or organoleptic data. The approach was useful for determining the direction and degree of preference for different grass silages offered fresh or after 48 hours of oxygen exposure to pregnant Holstein heifers. Differences in palatability were observed based on the duration of oxygen exposure but not between the source of silage. Application of this methodology can be extended to other silage types or qualities when a paired comparison of palatability is desired. Recently, our group (Scuderi et al., 2025) characterized the impact of multi-strain inoculation of chopped whole plant corn at harvest on subsequent corn silage intake at desiling (0 hour) or after 24 hours of oxygen exposure using pregnant Holstein heifers. Although inoculant use had minimal impact on fermentation and nutritional composition of the silage, inoculation improved silage palatability as measure through differences in dry matter intake. This study demonstrated that application of inoculants can provide a benefit beyond the storage structure.

Conclusions

Some VOC may affect intake, metabolism, and performance of ruminants because of the VOC's chemical structure and sensory characteristics. Use of preference studies based on feeding behavior and DMI may help elucidate which type or quality of silage will affect DMI and ultimately performance. By understanding the factors that influence silage preference, silage ensiling and feed-out management practices can be improved supporting overall improvements in ruminant productivity.

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Performance of Holstein Cows Fed Diets Containing Corn Silage Treated with a Multi-strain Silage Inoculant

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Keywords: Corn silage, Inoculant, Energy-corrected milk, *Lentilactobacillus hilgardii*, *Lentilactobacillus buchneri*

Introduction

The use of microbial silage inoculants is a well-accepted practice for forage management programs, with current formulations including a combination of homofermentative and heterofermentative lactic acid bacteria (LAB) strains. Homofermentative strains are effective in rapidly decreasing the pH during the earlier phases of ensiling (Muck et al., 2018), whereas heterofermentative strains produce multiple organic acids and end-products, including acetate and 1,2-propanediol, which have been postulated to improve stability during aerobic feed-out (Driehuis et al., 1999). Additionally, the use of effective LAB strains to aid in a rapid pH decrease and improve aerobic stability also helps reduce dry matter (DM) losses (Borreani et al., 2018). Such improvements have implications for improving the management of forage inventory available for feeding. Furthermore, improvements in fermentation quality by inoculation can have implications on animal performance. Some research has demonstrated increased milk production in response to improved silage fermentation; however, further work is needed to characterize the mode of action(s) (Oliveira et al., 2017). Therefore, the objective of this study was to evaluate the lactation performance of Holstein cows offered diets containing corn silage that was either treated or not at harvest with a multi-strain silage inoculant.

Materials and methods

Holstein cows ($n = 46$; 83 ± 25 d in milk; 2.2 ± 1.2 lactations) were enrolled in a randomized, complete block design study with a 2-wk covariate period and a 9-wk treatment period to evaluate diets containing 50% corn silage (CS) inoculated with a multi-strain inoculant or not at harvest. Treatments were a diet with: 1) non-inoculated CS (CON) and 2) CS inoculated with Magniva Platinum 3 (MSI; Lallemand Specialties Inc., Milwaukee, WI; *Lentilactobacillus buchneri*, *Lentilactobacillus hilgardii*, and *Pediococcus pentosaceus* at 2.5×10^5 cfu/g forage; β -glucanase at 5750 IU/g; xylanase at 30000 IU/g). Chopped whole plant corn was ensiled by inoculant treatment in individual bunkers (19.8 m x 6.1 m x 2.0 m) for ~250 d before use. Corn silage from each bunker was removed using the same rate daily (~20 cm/d) to ensure similar oxygen exposure and was weighed and classified as follows: 1) material removed and of quality to feed and 2) spoiled/not of quality to feed. Weight data were used to calculate losses from fermentation and spoilage. The diets on a DM basis contained 57% forage, 16% crude protein, 30% neutral detergent fiber, and 28% starch and were fed as a TMR. The CS was replaced 1:1 on a DM basis. Cows were group-housed in a freestall pen, fed individually 1x/d with a Calan Broadbent Feeding System (American Calan, Northwood, NH), milked 3x/d, and milk sampled 6x/wk. One cow was removed from the final dataset because of failure to respond to treatment for mastitis. Data from 45 cows were analyzed by ANCOVA (MIXED procedure of SAS v 9.4). The model included a covariate and contained fixed (i.e., block, diet, week, interaction) and random (i.e., cow) effects.

Results

The CS had similar nutrient profiles and digestibilities but different fermentation profiles with a higher concentration of acetate in the MSI CS (Table 1). Furthermore, the MSI CS had more good quality (i.e., not heated or moldy) material available for feeding than the CON CS (Table 2). However, only good quality silage was incorporated into the TMR for both treatment groups. Dry matter intake (DMI), milk yield, milk composition, and feed efficiency (Table 3) were not affected by diet ($P > 0.10$) but were affected by week ($P \leq 0.01$). The interaction of diet by week was not significant ($P > 0.10$). In a case scenario using data from this study, energy-corrected milk (ECM) yield (kg) per material ensiled (kg dry matter) incorporated into a balanced diet was greater for MSI CS (2.98) than CON CS (2.50) when fermentation and spoilage losses were considered.

Table 1. Composition of corn silage measured during the treatment period of the study that was not (CON CS) or was inoculated with a multi-strain inoculant (MSI CS) at harvest.

	CON CS	MSI CS
Crude protein, % of dry matter (DM)	6.6±0.4 ¹	6.7±0.2
Starch, % of DM	38.8±1.4	39.0±0.8
Neutral detergent fiber (NDF), % of DM	37.3±1.1	38.9±0.9
7-h starch digestibility, % of starch	75.3±4.2	76.3±4.1
30-h NDF digestibility, % of NDF	59.3±2.5	58.7±1.4
Lactic acid, % of DM	4.6±0.2	1.5±0.2
Acetic acid, % of DM	1.7±0.3	3.5±0.5
pH	3.9±0.0	4.2±0.0

¹ Mean ± standard deviation.

Table 2. Ensiling and desiling characterization of corn silage (CS) that was not (CON) or was inoculated with a multi-strain inoculant (MSI) at harvest.

	CON CS	MSI CS
Material ensiled, as-fed metric ton	95.01	91.98
Material removed, as-fed metric ton	91.20	88.89
Not spoiled and of quality to feed	65.10	74.33
Spoiled/not of quality to feed	26.10	14.56
Calculated spoilage/not of quality to feed, % of material removed	28.6	16.4

Table 3. Lactational performance of cows fed diets containing corn silage that was not (CON) or was inoculated with a multi-strain inoculant (MSI) at harvest.

	CON Diet	MSI Diet	SE	Treatment	P-value	
					Time	Treatment x Time
Dry matter intake (DMI), kg/d	25.7	25.4	0.2	0.34	<0.001	0.19
Energy-corrected milk (ECM), kg/d	46.9	46.8	0.5	0.95	<0.001	0.45
Fat, kg/d	1.64	1.63	0.03	0.84	<0.001	0.23
True protein, kg/d	1.35	1.35	0.01	0.99	<0.001	0.61
ECM/DMI	1.81	1.85	0.02	0.14	<0.001	0.11

Conclusions

Although the use of a *L. buchneri*, *L. hilgardii* and *P. pentosaceus*-based corn silage inoculant did not affect lactation performance of high-producing dairy cows, the application of an inoculant at harvest was still beneficial based on the reduced spoilage and heating of material in the bunker.

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Preservation of High Moisture Male Sterile BMR Forage Sorghum Silage

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Keywords: Dry matter loss, male sterile BMR sorghum, microbial inoculant

Introduction

Male sterile brown midrib (BMR) forage sorghum is a crop that has been shown to improve forage digestion (Hao et al., 2021). The BMR trait, coupled with male sterility, is unique in that it can enhance the nutritional value of the forage sorghum. Although there are benefits to enhanced nutritional value, there are challenges associated with nutrient preservation during ensiling, particularly when the crop is harvested at a high moisture content. This high moisture is beneficial to achieve proper fermentation but can be detrimental due to clostridia and butyric acid formation. Inoculants can be utilized to prevent these outcomes. The two main types of inoculants available are homofermentative and heterofermentative (Muck, 2010). This study was conducted to discern differences between inoculant types when ensiling high moisture male sterile BMR forage sorghum.

Materials and Methods

The study was conducted at the University of Tennessee research farm on June 6th 2020 and evaluated the effects of different inoculants on male sterile BMR forage sorghum silage. The target seeding rate was 8.97 kg/ha. Unfortunately, the older drill that was used planted half at the correct rate and half at a much higher rate, which had significant lodging. The part that grew normal, not lodged, was harvested, starting when most of the field had headed, and continued each Tuesday for 10 weeks. At week 1 (just headed), week 5 (halfway to optimum), and week 9 after heading to optimize nutrient concentration in forage cells and to duplicate corn silage, which is chopped 56 days after tassel. The treatments were water mist for control (CON), homofermentative inoculant (MC, (*Lactiplantibacillus plantarum* (DSM16568), *Enterococcus lactis* (DSM22502) and *Lactococcus lactis* (NCIMB30117)) SiloSolve® MC, Novonesis, Lyngby, Denmark) or heterofermentative inoculant (FC, (*Lactococcus lactis* (DSM11037) and *Lentilactobacillus buchneri* (DSM22501), SiloSolve® FC, Novonesis, Lyngby, Denmark). Inoculants were applied at 150,000 CFU/g of fresh matter. Upon harvest, 6 individual typical plants were cut, chopped in a portable chopper, and treated with either SiloSolve® MC or FC. The 6 control samples were treated with misted water only. Each sample was vacuum-sealed and fermented for 3 weeks before analysis. The samples were then sent to Cumberland Valley Analytical Services for NIR+, wet chemistry starch and sugar, and fermentation analysis. Statistical analysis was performed by comparing treatment groups by sampling weeks, treating each sample as its own experimental unit without repeated measures. A multiple range test was used to test for significance ($P < 0.05$).

Results

An unexpected factor arose 5 weeks after heading when the sorghum variety sent out lateral shoots with male sterile heads. This factor began influencing most nutritional components during the last two harvest dates. For the weeks after this, it drastically altered the nutrient enhancement as the plant utilized photosynthetic products to produce the lateral shoots instead of increasing the digestible components of the plant. An example is the WSC sugar, which recorded a 33% drop from weeks 5 to 9.

The lateral shoot production also resulted in a forage that did not increase in dry matter like previous research had shown. In the first week of the study, the forage dry matter was just over 17% for the treated and control samples, which is typical for sorghum at this stage. In the second set of samples at week 5, the dry matter had increased to just under 25%. The multi-head kept the crop from increasing dry matter, which was only 27.6% dry matter at the end of the study.

When looking at preserved forage quality, the FC was significantly lower than the MC and the control for Non-Fiber Carbohydrate and Non-Structural Carbohydrate. The FC treatment group was significantly lower for the Water-Soluble Carbohydrates, where much of the energy of male sterile BMR sorghum resides. In fact, at the final harvest – where most farmers would be chopping – the MC had 2.6 times more sugar than the FC. The control was similar to MC. At all three harvest dates, the MC had lower pH. It was significantly lower in weeks 5 and 9 than in the control and the FC. In the early wetter harvest, the MC had the most Total VFA. As the crop developed more sugars and became drier, the FC was significantly higher in Total VFA than in the other two treatments. The MC and control were significantly higher than the FC in Lactic (%) in terms of both the percentage of dry matter and the percentage of total VFA. Acetic acid plays a critical role in secondary fermentation prevention. FC was nearly 6 times higher here than the MC and the control.

Table1. Characteristics of male sterile BMR forage sorghum (n=6 per treatment/sampling) prepared without inoculation (CON), treated with SiloSolve FC (FC), or SiloSolve MC (MC).

	Week 1			Week 5			Week 9		
	FC	MC	Control	FC	MC	Control	FC	MC	Control
Dry Matter (%)	17.47	17.55	17.82	23.75	25.05	24.52	25.63	27.2	27.38
NDF (%)	66.9	65.22	63.3	52.58 ^a	49.68 ^b	51.12 ^{ab}	59.13 ^a	56.42 ^b	56.7 ^b
NDFd-30-NDF (%)	62.48 ^a	61.82 ^{ab}	61 ^b	55.9 ^a	58.35 ^b	56.42 ^a	48.03 ^a	45.78 ^b	48.1 ^a
Starch-NFC (%)	1.23	1.37	1.26	9.62	8.17	10.58	11.97	9.22	9.33
TDN	62.97 ^a	63.95 ^b	63.4 ^{ab}	65.28 ^a	67.57 ^b	65.72 ^a	60.2	60.35	60.78
NFC (%)	16.4 ^a	18.42 ^{ab}	20.1 ^b	30.12 ^a	37.2 ^b	34.32 ^b	28.6 ^a	31.93 ^b	31.85 ^b
NSC (%)	4.93 ^a	7.35 ^b	8.74 ^c	7.2 ^a	18.55 ^b	14.67 ^c	9.15 ^a	13.82 ^b	14.22 ^b
pH	4.05 ^a	3.68 ^b	4.03 ^a	3.89 ^a	3.37 ^b	3.56 ^c	3.74 ^a	3.56 ^b	3.65 ^c
TotalVFA (%)	7.39 ^a	10.2 ^b	7.96 ^a	9.49 ^a	7.66 ^b	6.91 ^b	8.19 ^a	7.34 ^b	6.1 ^b
Lactic -TotVFA (%)	41.12 ^a	95 ^b	89.6 ^c	48 ^a	95 ^b	73.67 ^c	45.5 ^a	89.8 ^b	86 ^b
Lactic (%)	3.05 ^a	9.68 ^b	7.14 ^c	4.55 ^a	7.27 ^b	5.1 ^a	3.7 ^a	6.58 ^b	5.25 ^c
Acetic (%)	4.34 ^a	0.52 ^b	0.82 ^b	4.94 ^a	0.39 ^b	1.81 ^c	4.49 ^a	0.76 ^b	0.85 ^b
Water Soluble CHO sugar	2.21 ^a	4.58 ^b	5.97 ^b	5.83 ^a	22.81 ^b	15.38 ^c	4.54 ^a	12.06 ^b	12.14 ^b

Values in rows with different superscript letters are different at $P < 0.05$ within each sampling week.

Conclusions

Although this study had challenges, the inoculated forage still showed beneficial fermentation characteristics. The Novonesis inoculants could handle this wetter forage and properly ferment the crop given the pH that was exhibited here. For this forage, it is critical to identify the right genetic strain for a given area to prevent the production of the shoots exhibited in this study.

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Citrus Essential Oil Effects on Chemical Composition and *In Vitro* Degradation of Sugarcane Silage

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Keywords: d-limonene, neutral detergent fiber, nutritional value, orange.

Introduction

Livestock requires a forage allowance yearly to improve productivity and reduce environmental impact. In Brazilian conditions, sugarcane has been used to produce more than other crops with an editable nutritional value (Cantoia Jr., et al., 2020). However, sugarcane conservation by ensiling has significant challenges, such as low dry matter content and high water-soluble carbohydrate content, which favor the growth of epiphytic yeasts that generally promote an alcoholic fermentation (Cantoia Jr. et al., 2020).

In this context, additives and inoculants can improve silage fermentation and nutritional value. Many additives, such as chemicals and essential oils (EO) can have antifungal effects and improve sugarcane silage preservation (Cantoia Jr. et al., 2020). Besides traditional EO used in animal feeding and evaluated as ensiling additives, sweet orange or orange tree (*Citrus sinensis* (L.) Osbeck) is a highly available EO. This EO has more than 300 different chemical compounds, and those in greatest abundance are limonene (94 %), α -pinene (0.54 %), sabinene (0.74 %), and β -myrcene (1.18 %). Limonene is primarily responsible for the oil's antifungal properties (Marriott et al., 2001), making sweet orange oil a promising candidate as a silage inoculant.

Silva et al. (2024) evaluated increasing levels of citrus EO. They observed a linear increase in corn silage dry matter and organic matter content, with no significant effect on *in vitro* degradation. However, silage environmental conditions and EO extraction methods can greatly affect essential oil impact on silage preservation. To the best of our knowledge, no study has evaluated the effects of orange EO addition during the sugarcane ensiling. Therefore, we hypothesized that citrus EO reduces fiber content and improves *in vitro* degradation especially when essential oils were obtained by distillation. This study aimed to evaluate the citrus EO effect on chemical composition and *in vitro* degradation of sugarcane silage.

Materials and methods

Two trials were performed at GETAP the Centro de Ciências Agrárias of Universidade Federal de São Carlos (UFSCar). In each trial, thirty-two experimental silos were used in a blocked-randomized design to evaluate the following treatments: 0, 250, 500 and 750 mg of citrus essential oil/ kg DM of fresh sugarcane. Trial 1 used an essential oil obtained by pressing and silos storage was performed for 60 days. Trial 2 used EO obtained by liqueur distillation and storage was performed for 100 days. After opening the silos, central samples were collected and followed protocols (Cantoia Jr. et al., 2020) for evaluations.

Results

Citrus EO addition in sugarcane silage linearly ($P \leq 0,011$) increased ADF content without affecting *in vitro* degradation of DM and NDF in trial 1 (Table 1). However, in trial 2, citrus EO did not affect the chemical composition and linearly increased ($P \leq 0,025$) DM and NDF *in vitro* degradation.

Table 1. Chemical composition and in vitro degradation of sugarcane silage with increasing levels of d-Limoneno (essential oil extracted

by pressing orange pills). Ensiling period was 60 days

¹Increasing levels of citrus essential oil (0, 250, 500, and 750 mg/kg DM);

²Standard error of mean;

³Probabilities: Treat.: Treatments effect; linear (lin.), quadratic (Qua.), and quadratic deviation (Des.) effect of citrus EO.

Item	Treatments ¹				SEM ²	Probabilities ³			
	0	250	500	750		Treat.	Lin.	Qua.	Des.
TRIAL 1									
Chemical composition, g/kg dry matter, unless stated									
Dry matter, g/kg as-fed	161	160	157	157	0.18	0.037	0.006	0.542	0.584
Organic matter	957	960	953	956	0.14	0.079	0.187	0.903	0.024
Neutral detergent fiber	689	711	700	718	0.66	0.200	0.095	0.855	0.174
Acid detergent fiber	387	393	389	406	0.35	0.025	0.011	0.259	0.145
Crude protein	39.4	38.7	41.2	40.0	0.13	0.234	0.312	0.780	0.084
<i>In vitro</i> degradation, g/kg									
Dry matter	449	422	434	432	0.73	0.463	0.461	0.312	0.321
Neutral detergent fiber	199	187	192	208	1.08	0.826	0.662	0.411	0.948
TRIAL 2									
Chemical composition, g/kg dry matter, unless stated									
Dry matter, g/kg as-fed	183	178	177	184	0.41	0.487	0.803	0.134	0.824
Organic matter	970	969	971	972	0.11	0.487	0.192	0.538	0.574
Neutral detergent fiber	627	630	638	612	0.86	0.326	0.389	0.156	0.385
Acid detergent fiber	363	368	363	354	0.74	0.409	0.240	0.241	0.782
Crude protein	34.4	34.8	35.4	34.6	0.17	0.785	0.737	0.415	0.610
<i>In vitro</i> degradation, g/kg									
Dry matter	484	526	537	542	1.34	0.015	0.004	0.159	0.650
Neutral detergent fiber	171	249	247	253	1.93	0.050	0.025	0.117	0.368

Conclusions

Citrus EO can increase the nutritional value of sugarcane silage. However, EO impact depends on environmental silage conditions.

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Mineral Contents of Sweet Corn Stover Silage Effect of Mechanical Processing and Ensiling Duration

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Keywords: feed, maize, ruminant

Introduction

In the tropics, minerals are vital to the health and welfare of ruminant animals since both their excess and shortage may result in low productivity (Dele et al., 2021). Corn stover being a by-product of maize production, is considered a valuable source of feed because of its relative abundance (Methu et al., 2001) and often fed as sole forage. Heuzé et al. (2019) reported that corn stover is a good source of minerals for ruminants when freshly harvested. Mtengeti et al. (2014) reported that chopping of grasses lead to increased crude mineral content. This study was design to assess the mineral contents of sweet corn stover silage as influence by processing methods (chopping and shredding) and ensiling duration (0, 4, 8 and 16 weeks after ensiling).

Materials and methods

In October 2023, sweet corn stover was harvested from a commercial farm (Africa Steading Farms Ltd) Adewusi village Odeda, Ogun State, Nigeria. Sweet corn (Sugar-75 F1) was planted at 30cm intra-row and 100cm inter-row. The cobs were harvested on 67 days after planting while the stover was harvested on 2nd day after harvesting of cobs (68th day after planting) and was divided into two halves with a portion was chopped and another portion shredded. The chopped/shredded materials were ensiled in 25-liter silos, sealed and stored for 4, 8 and 16 weeks. The study was a 2 (processing: chopped and shredded) x 4 (ensiling period: 0, 4, 8 and 16 weeks after ensiling) factorial design with five (5) replicates. Post-ensiling, the silos were opened and silage samples were dried at 65 °C for 72 hours and analyzed for macro (Ca, P, K, Na, Mg) and micro minerals (Cu, Zn, Mn, Fe) using atomic absorption spectrophotometry and flame photometry. Data were analyzed using the general linear model of SAS computer package was used for the analysis and significant difference was tested at 5% probability level.

Results

The mineral contents of the silages were significantly ($P < 0.05$) influenced by the interaction of the processing method x ensiling duration. The Ca content ranged between 2.14 gkg⁻¹ DM for the chopped but unensiled forage to 7.07 gkg⁻¹ DM for the shredded ensiled foe 16 weeks. The unensiled fodders had higher P content than the ensiled with unensiled shredded having the highest P (3.40 gkg⁻¹ DM) content. The K content ranged from 6.09 gkg⁻¹ DM to 11.42 gkg⁻¹ DM with the chopped ensiled for 4 weeks having the highest value. The fodder chopped and ensiled for 8 weeks had the highest Fe (790.53 mgkg⁻¹ DM) and the least Mn and Zn contents of 9.51 mgkg⁻¹ DM and 13.60 mgkg⁻¹ DM, respectively whereas the unensiled shredded recorded the highest Mn and Zn (53.30 mgkg⁻¹ DM and 24.50 mgkg⁻¹ DM, respectively) contents.

Table 1: Effect of processing and ensiling duration on the mineral contents of sweet corn stover silage

Processing	Duration (WOE)	Ca	P	K	Mg	Na	Fe	Mn	Cu	Zn
gkg ⁻¹ DM						mgkg ⁻¹ DM				
Chopped	0	2.14b	3.09b	6.09g	3.95e	1.59bcd	121.12g	52.78a	0.72g	22.36b
	4	3.08b	1.68g	11.42a	4.46b	0.66e	207.26f	26.03f	1.75d	14.57f
	8	3.12b	2.24e	9.23d	3.57g	2.03ab	790.53a	9.51g	2.05b	13.60g
	16	2.57b	3.08b	9.29d	3.97e	1.43cd	471.74b	44.81b	1.59e	19.93c
Shredded	0	6.59a	3.40a	7.74e	4.32c	1.09de	383.12c	53.30a	1.49f	24.50a
	4	2.75b	2.11f	9.58c	4.62a	0.76e	322.86d	38.91c	1.89c	17.73e
	8	2.97b	2.74c	7.01f	4.21d	2.40a	260.50e	31.48e	0.66h	18.26d
	16	7.07a	2.53d	10.33b	3.79f	1.70bc	785.19a	37.37d	2.36a	19.86c
SEM		0.32	0.06	0.19	0.04	0.09	26.54	1.54	0.06	0.39
P-Value										
Processing (P)		<0.0001	<0.0001	<0.0001	<0.0001	0.6447	<0.0001	<0.0001	<0.0001	<0.0001
Duration (D)		0.0201	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P x D		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

a, b, c, ...h: Means in same column with different letters were significantly ($P < 0.05$) different. SEM= Standard error of means, WOE= weeks after ensiling

Conclusions

Mineral content in silages was greatly influenced by the interaction of processing method and ensiling duration. Ensiling reduced P, Mn, and Zn while increasing Ca and K. Treatment-specific variations in optimal mineral profiles underscore the significance of ensiling duration and processing techniques in silage production.

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PROTEIN FRACTIONS OF MEGATHYRSUS-UNALABLE ORANGE SILAGE

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Keywords: citrus, conservation, grass

Introduction

In the tropics, ruminant livestock productivity is severely impacted by seasonal feed scarcity (Lawal-Adebawale, 2020). Smallholder farmers rely heavily on natural pastures, which are abundant during the rainy season but become fibrous and less valuable during the dry season. Excess herbage can be conserved as silage, providing a valuable feed resource during scarcity periods. Additionally, utilizing perishable fruits, which account for 40-60% of post-harvest food losses in Nigeria (FMARD, 2016), as silage can lessen the negative effects on the environment. This study evaluates the protein fractions of silage mixtures composed of *Megathyrsus maximus* and unsalable orange for smallholder ruminant livestock systems, providing a novel approach to mitigating feed scarcity and reducing waste.

Materials and methods

The study was carried out at the Pasture Unit, Directorate of University Farms, Federal University of Agriculture, Abeokuta, Nigeria. *Megathyrsus maximus* was sourced from the wild within the University, and a high-density portion was cut back to 10 cm above ground level, allowed to regrow, and harvested after 8 weeks at 15 cm above ground level. The grass was chopped using a JF-2D fodder processor and wilted for 4 hours. The unsalable orange used for this study was sourced from a local fruit market less than 10km from the university. The orange pomace was prepared by extracting juice with the aid of a hydraulic press, chopped and wilted for 8 hours. Forage mixtures included sole *M. maximus* (control) and *M. maximus* + unsalable orange in ratios of 90:10, 80:20, and 60:40. The materials were ensiled in 960 ml laboratory silos, sealed and stored for 3, 6 and 9 weeks in a factorial design of 4 forage mixtures × 3 ensiling periods with 6 replicates. Post-ensiling, silage samples were oven dried at 55°C for 72 h and milled. The milled samples were analyzed for crude protein (CP) contents were determined according to AOAC (1990). The crude protein fractions of the samples were estimated following procedure of Sniffen et al. (1992) modified by Licitra et al. (1996). Data were analyzed using the general linear model of SAS computer package was used for the analysis and significant difference was tested at 5% probability level.

Results

The forage mixture × ensiling duration significantly ($P < 0.0001$) influenced the crude protein (CP) and the protein fractions of the silages. The CP of the silage produced from forage mixture of 60% Megathyrsus + 40% unsalable orange ensiled for 3 weeks was the highest (118.75 gkg⁻¹ DM). The silage produced from forage mixture of 60% Megathyrsus + 40% unsalable orange ensiled for 3 weeks also had the highest values for A, B2, B3 and C (51.04, 19.92, 29.18 and 18.19 gkg⁻¹ DM, respectively) fractions. The silage produced from forage mixture of 90% Megathyrsus + 10% unsalable orange ensiled for 3 weeks had the highest B1 (3.61 gkg⁻¹ DM) fraction.

Table 1: Effect of ensiling duration on the protein fractions (g/kg-1 DM) of unsalable orange-Megathyrus silage

Forage mixture (%)	Ensiling Duration (WOE)	CP	A	B1	B2	B3	C
M100	3	109.54 ^{bcd}	47.86 ^{bcd}	3.38 ^{bcd}	17.69 ^{bc}	25.92 ^{bcd}	15.95 ^g
	6	106.91 ^{bcde}	46.88 ^{bcde}	3.48 ^{ab}	17.06 ^{bcd}	25.00 ^{bcde}	14.74 ^h
	9	104.74 ^{ef}	46.17 ^{ef}	3.44 ^{bcd}	16.25 ^{de}	24.23 ^{ef}	16.84 ^{de}
M90+U10	3	110.91 ^b	48.48 ^b	3.61 ^a	18.05 ^b	26.40 ^b	17.91 ^{ab}
	6	91.83 ⁱ	41.12 ⁱ	3.20 ^e	13.39 ^h	19.65 ^j	16.86 ^{de}
	9	96.89 ^h	42.90 ^h	3.29 ^{cde}	14.61 ^g	21.42 ^h	16.73 ^{ef}
M80+U20	3	100.41 ^{gh}	44.62 ^g	3.28 ^{de}	15.49 ^{fg}	22.69 ^{gh}	17.27 ^{cd}
	6	106.39 ^{cde}	46.52 ^{cdef}	3.49 ^{ab}	16.87 ^{cde}	24.81 ^{cde}	16.30 ^{fg}
	9	105.45 ^{def}	46.34 ^{def}	3.45 ^{bc}	16.72 ^{cde}	24.50 ^{def}	16.21 ^g
M60+U40	3	118.75 ^a	51.04 ^a	3.42 ^{bcd}	19.92 ^a	29.18 ^a	18.19 ^a
	6	110.45 ^{bc}	48.09 ^{bc}	3.54 ^{ab}	17.92 ^b	26.23 ^{bc}	16.74 ^{ef}
	9	102.03 ^{fg}	44.92 ^{fg}	3.38 ^{bcd}	15.87 ^{ef}	23.25 ^{fg}	17.69 ^{bc}
SEM		0.95	0.35	0.02	0.23	0.34	0.12
FM		<0.0001	<0.0001	0.2796	<0.0001	<0.0001	<0.0001
ED		<0.0001	<0.0001	0.6327	<0.0001	<0.0001	<0.0001
FM x ED		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

a, b, c, ...i: Means in same column with different superscripts were significantly ($P<0.05$) different. CP = Crude protein, A = Non Protein Nitrogen, B₁ = Soluble true protein, B₂ = Soluble protein in neutral detergent, B₃ = Insoluble protein in neutral detergent but soluble in acid detergent, C = Unavailable protein/ True protein, SEM= Standard error of means, WOE= weeks after ensiling, M= Megathyrus maximus, U= Unsalable orange

Conclusions

It was concluded that 60% *M. maximus* + 40% unsalable orange silage showed higher CP content as well as the higher A fraction could ensures immediate ruminal protein availability, supporting microbial growth and fermentation and suggest that there will be enhanced nitrogen utilization efficiency. The 90% *M. maximus* + 10% unsalable orange silage's greater B1 fraction indicates a more soluble protein supply, which could be advantageous for ruminants especially high producing ones that need easily accessible nitrogen for quick growth. Lastly, forage mixtures and ensiling duration can optimize protein supply for ruminants, improving growth performance, milk production, and overall feed efficiency.

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Effect of Cutting Height on Nutritive Value, Dry Matter Yield, and Fermentation Profile of Whole-plant Corn Forage and Silage: A meta-analysis

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Keywords: corn, cutting height, starch

Introduction

Whole-plant corn silage (WPCS) is an important component of dairy cow rations, since it provides energy to support lactation and physically effective fiber to support rumen health. Although there are many management strategies available for dairy farmers to manipulate the nutritive value of corn silage, adjusting cutting height at harvest can give farmers flexibility to improve nutritive value or pursue greater yields (Ferraretto et al., 2018). However, increasing cutting height improves nutritive value at the expense of DM yield (Ferraretto et al., 2018), which can create difficult decisions for producers. Although there are many trials that have evaluated the effect of cutting height on corn forage and silage and others have reviewed the effect of cutting height in literature (Wu and Roth, 2003), no attempts have been made to quantify observed results in literature. Thus, the objective of this study was to perform a meta-analysis using literature data on the effects of cutting height in WPCS or whole-plant corn forage (WPCF) on nutritive value, DM yield, and fermentation profile.

Materials and methods

A comprehensive search of literature published from 2000 to 2024 was conducted to identify studies evaluating the effect of cutting height on the nutrient composition, DM yield, and fermentation profile of WPCS and WPCF. A literature search was conducted using Google Scholar, PubMed, and ScienceDirect with the keywords “corn silage” or “corn forage” + “cutting height” or “chop height.” Thirty-seven publications were assessed for eligibility, and one was excluded due to not being published in English or Portuguese, while another was excluded because it did not report NDF concentration. Data from 35 peer-reviewed manuscripts, with a total of 151 observations, met the selection criteria of this meta-analysis. The dependent variables evaluated were WPCS/WPCF nutrient composition, DM yield, and fermentation profile. Data were analyzed using the mixed procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The model included the fixed effects of cutting height and the random effect of treatment (other treatments not including cutting height) within study. Both linear and quadratic effects of cutting height were evaluated for each variable. Observations were weighted with the inverse of the standard error of the mean (SEM; $1/SEM$) for response variables per treatment (other treatments not including cutting height) within study according to Roman-Garcia et al. (2016). Statistical significance was declared at $P \leq 0.05$.

Results

No quadratic effects were observed ($P > 0.05$) for any variables. A linear relationship between cutting height and DM concentration was observed ($P = 0.001$). For every 10 cm increase in cutting height, DM concentration increased approximately 0.5 percentage-units. A linear effect between cutting height and CP concentration was observed ($P = 0.001$). For every 10 cm increase in cutting height, CP concentration increased by 0.1 percentage-units. An inverse linear relationship between cutting height and NDF concentration was observed ($P = 0.001$). For every 10 cm increase in cutting height, a 0.9 percentage-unit decrease in NDF concentration was observed. An inverse linear relationship between cutting height and ADF concentration was observed ($P = 0.001$). The concentration of ADF decreased by 0.8 percentage-units for every 10 cm increase in cutting height. A linear relationship between cutting height and NDFD was observed ($P = 0.001$). For every 10 cm increase in cutting height, NDFD increased by 0.8 percentage-units. Inverse linear relationships between cutting height and lignin concentration ($P = 0.001$) as well as uNDF concentration and cutting height ($P = 0.01$) were observed. Lignin concentration decreased 0.1 percentage-units for every 10 cm increase in cutting height, while the concentration of uNDF decreased by 0.5 percentage-units. A linear relationship between starch concentration and cutting height was observed ($P = 0.001$). Starch concentration increased by 0.9 percentage-units for every 10 cm increase in cutting height. An inverse linear relationship between ash concentration and cutting height was observed ($P = 0.001$). Ash concentration decreased by 0.1 percentage-units for every 10 cm increase in cutting height. An inverse relationship between DM yield and cutting height was observed ($P = 0.001$). For every 10 cm increase in cutting height, DM yield decreased by 0.6 Mg/ha. No linear or quadratic effects were observed ($P > 0.05$) on pH, lactic acid, or acetic acid.

Table 1. Linear relationships between cutting height and nutrient composition and fermentation profile of whole-plant corn silage or forage.

Item ¹	Intercept	SE	Slope	SE	P - Value
DM, % as fed	33.3	0.48	0.05	0.01	0.001
CP, % as fed	7.5	0.17	0.01	0.001	0.001
NDF, % DM	48.2	0.81	-0.09	0.01	0.001
NDFD, % NDF	54.8	2.08	0.08	0.02	0.001
ADF, % DM	26.1	0.50	-0.08	0.004	0.001
Lignin, % DM	3.7	0.22	-0.01	0.002	0.001
uNDF, % DM	12.4	0.98	-0.05	0.01	0.01
Starch, % DM	25.6	1.09	0.09	0.01	0.001
Ash, % DM	4.3	0.22	-0.01	0.002	0.001
DM Yield, Mg/ha	17.3	0.76	-0.06	0.01	0.001
pH	3.82	0.03	0.0001	0.001	0.89
Lactic Acid, % DM	5.20	0.32	-0.01	0.004	0.13
Acetic Acid, % DM	1.03	0.13	-0.001	0.002	0.63

¹DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; NDFD: neutral detergent fiber digestibility; ADF: acid detergent fiber; uNDF: undigested neutral detergent fiber.

Conclusions

As expected, increasing cutting height of WPCS or WPCF improved nutritive value by increasing starch concentration and NDFD at the expense of DM yield. Additionally, it appears that overall altering cutting height does not affect fermentation in WPCS.

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Effect of Packing Density, Microbial Inoculation, and Storage Length on the Fermentation Profile and Nutrient Composition of Alfalfa Silage

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Keywords: alfalfa haylage, homofermentative, heterofermentative

Introduction

Alfalfa silage is typically wilted to reduce the chance of clostridial fermentation. However, wilted silage that is too dry or too coarsely chopped may be difficult to properly pack, increasing storage losses and negatively affecting fermentation (Rooke and Hatfield, 2003). Homofermentative inoculants improve fermentation profile and lactic acid production (Oude Elferink et al., 2001), which may decrease storage losses and curtail undesirable fermentation in poorly packed alfalfa silage. Alternatively, Liu et al. (2024) observed lower aerobic stability in poorly packed silage, meaning heterofermentative inoculants may decrease losses at feedout (Oude Elferink et al., 2001). The hypothesis of this study was that lower packing density would negatively affect the fermentation profile of alfalfa silage. Additionally, we hypothesized that inoculation with a homofermentative inoculant would increase lactic acid production, while inoculation with a heterofermentative inoculant would increase the production of acetic acid and aerobic stability.

Materials and methods

Alfalfa forage from the third cutting of the year was obtained from four locations (block) from the UW-Madison Arlington Agricultural Research Station after wilting for 48 h. Microbial inoculant treatments were applied by hand using spray bottles and consisted of: (1) 150,000 CFU/g forage of *Lactiplantibacillus plantarum* DSM26571, *Lactococcus lactis* NCIMB30117, and *Enterococcus lactis* DSM22502 (LPEL), (2) 150,000 CFU/g forage of *Lactococcus lactis* DSM22501 and *Lentilactobacillus buchneri* DSM11037 (LB) or (3) 15 mL of distilled water (CON). After microbial inoculation, alfalfa forage was packed into 20 L experimental silos at two densities: full density (D100; 264 kg of DM/m³) and half density (D50; 132 kg of DM/m³). Then experimental silos were randomly assigned to storage lengths of 7, 14, 60, or 90 d. Samples were evaluated for microbial counts and DM concentration. Additionally, samples were sent to a commercial laboratory for fermentation profile and nutrient composition analysis. Data were analyzed using PROC GLIMMIX of SAS with fixed effects of packing density, microbial inoculation, and storage length, with block as the sole random effect. Interaction effects were partitioned using the SLICE option and significance was declared when $P \leq 0.05$.

Results

An interaction between microbial inoculation and storage length was observed ($P = 0.001$; Figure 1A) for the pH of alfalfa silage. Overall, the pH of alfalfa silage in this experiment was high (> 4.9). At 7 and 14 d of storage, LPEL had the lowest pH (4.98 and 5.12, respectively), but no differences among treatments were observed at 60 and 90 d of storage. The pH of D50 was also 0.03 units greater ($P = 0.01$; Figure 1A) than D100. A three-way interaction was observed ($P = 0.03$; Figure 1B) for lactic acid concentration. Full density treatments generally had greater lactic acid concentration than D50, and D100-LB and D100-LPEL had the greatest concentration at 7 and 14 d of storage, respectively. An interaction between packing density and microbial inoculation was observed ($P = 0.02$; Figure 1C) for acetic acid concentration, where D100-CON and D100-LB (4.2% DM, on average) had greater concentrations than D50-LPEL and D50-LB (3.6% DM, on average). A three-way interaction was observed ($P = 0.001$; Figure 1D) for ammonia-N concentration, which was greater for D50 treatments than D100 treatments at later storage lengths. No butyric acid was detected in any treatments in this experiment. Ethanol concentration was 0.02 percentage-units greater ($P = 0.001$) for D100 than D50. No effects of any treatments in this experiment were observed ($P > 0.05$) on 1,2-propanediol or aerobic stability. Lactic acid bacteria counts decreased ($P = 0.001$) from 8.92 log CFU/g, on average, at 7 and 14 d of storage to 7.38 log CFU/g at 90 d of storage. An interaction between packing density and microbial inoculation was observed ($P = 0.01$) for DM losses and D100-LB (1.3% DM) had greater losses than D50-CON (1.0% DM). A three-way interaction was observed ($P = 0.001$) for SCP concentration. At 90 d of storage, D100-LB (62.9% CP) had a greater concentration of SCP than D50-LPEL (59.8% CP), but no other treatments differed from each other.

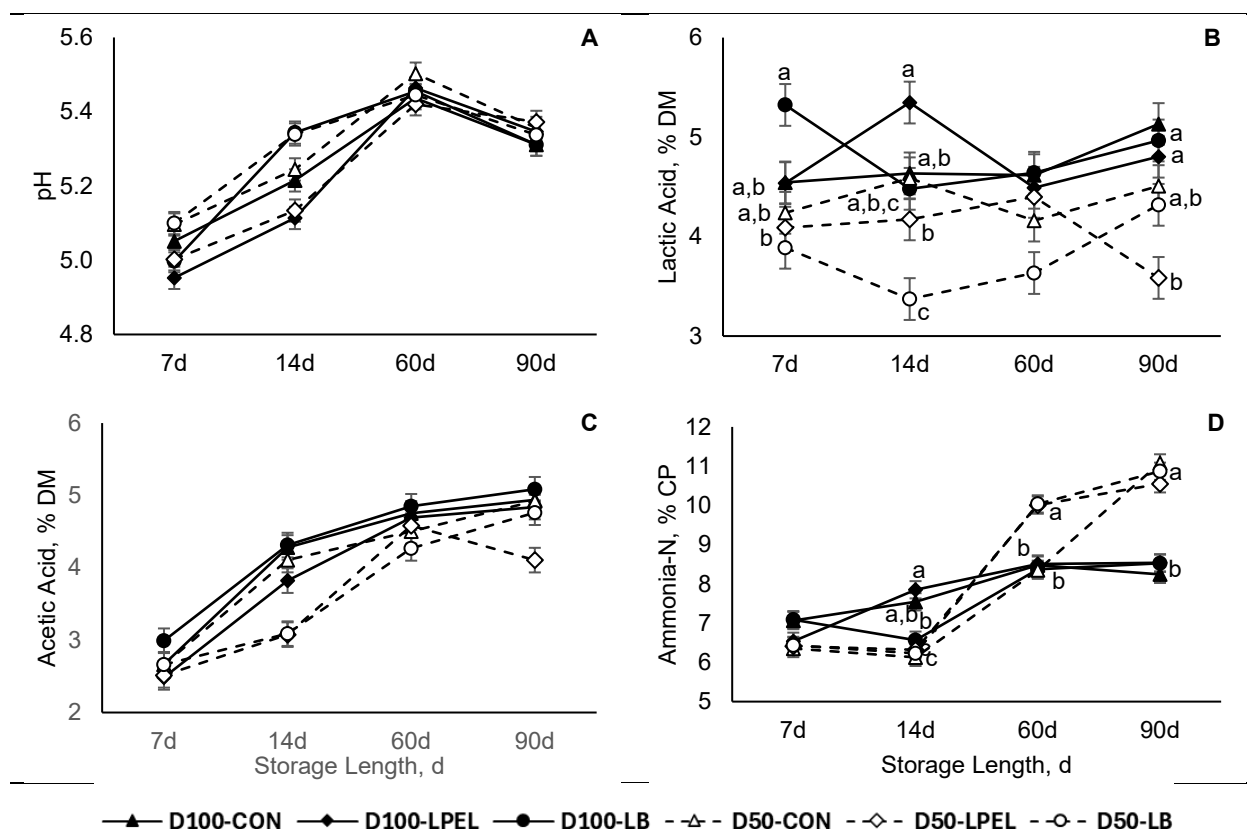


Figure 1. The effect of packing density, microbial inoculation, and storage length on pH, lactic acid, acetic acid, and ammonia of alfalfa silage. Treatments include packing density: D100 (full packing density) or D50 (50% of full packing density); inoculation: CON (distilled water), LPEL (*Lactiplantibacillus plantarum*, *Lactococcus lactis*, and *Enterococcus lactis*; 150,000 CFU/g forage), or LB (*Lactococcus lactis* and *Lentilactobacillus buchneri*; 150,000 CFU/g forage); storage length: 7, 14, 60 or 90 d. ^{a,b,c}Different superscripts denote differences among treatments within a storage length. (A) pH, $P = 0.58$; (B) Lactic acid (% DM), $P = 0.03$; (C) Acetic acid (% DM), $P = 0.22$; (D) Ammonia-N (% CP), $P = 0.001$.

Conclusions

Overall, microbial inoculants had minor effects on fermentation, although homofermentative inoculants appear to have slightly improved fermentation by decreasing pH and acetic acid concentration. Additionally, greater packing density improved fermentation by accumulating more organic acids and reducing ammonia-N concentrations along with storage losses.

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Effect of Pick-up Height During Merging, Microbial Inoculation, and Storage Length on the Fermentation Profile and Nutrient Composition of Alfalfa Silage

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Keywords: alfalfa haylage, soil contamination, homofermentative

Introduction

To decrease the chances of clostridial fermentation, producers often wilt alfalfa silage which can inadvertently increase the chance of soil contamination. However, clostridial fermentation can be initiated by soil contamination during harvest and packing (Queiroz et al., 2018). To promote desirable fermentation, crops like alfalfa may benefit from homofermentative inoculants, as a rapid acidification may curtail clostridial fermentation (Oude Elferink et al., 2001). Despite this, greater lactic acid production in homofermentative inoculants can promote yeast growth at opening (Oude Elferink et al., 2001), meaning heterofermentative inoculants may be beneficial to improve aerobic stability. The hypothesis of this study was that a lower pick-up height during merging would increase ash (soil) contamination and therefore promote an undesirable fermentation in alfalfa silage. Additionally, we hypothesized that inoculation with a homofermentative inoculant would curtail undesirable fermentation, while inoculation with a heterofermentative inoculant would increase the production of acetic acid.

Materials and methods

Alfalfa forage from the first cutting of the year was obtained from the UW-Madison Arlington Agricultural Research Station after wilting for 6 h. Before harvest, forage was merged at a pick-up height of 0 cm (LO) or 6 cm (HI) from four locations (block). Microbial inoculant treatments were applied by hand using spray bottles and consisted of: (1) 150,000 CFU/g forage of *Lactiplantibacillus plantarum* DSM26571, *Lactococcus lactis* NCIMB30117, and *Enterococcus lactis* DSM22502 (LPEL), (2) 150,000 CFU/g forage of *Lactococcus lactis* DSM22501 and *Lentilactobacillus buchneri* DSM11037 (LB) or (3) 15 mL of distilled water (CON). After microbial inoculation, alfalfa forage was packed into vacuum pouches and randomly assigned to storage lengths of 7, 14, 28, or 90 d. Samples were evaluated for microbial counts, DM, and ash concentration. Additionally, samples were sent to a commercial laboratory for fermentation profile and nutrient composition analysis. Data were analyzed using PROC GLIMMIX of SAS with fixed effects of pick-up height, microbial inoculation, and storage length, with block as the sole random effect. Interaction effects were partitioned using the SLICE option and significance was declared when $P \leq 0.05$.

Results

A three-way interaction was observed ($P = 0.001$; Figure 1A) for pH and across storage lengths, LO- and HI-LPEL had the lowest pH. Generally, HI pick-up height treatments also had greater pH than LO pick-up heights within a given inoculant. A three-way interaction was observed ($P = 0.05$; Figure 1B) for lactic acid concentration, where LO pick-up height treatments generally had greater lactic acid concentrations than HI pick-up heights and LO-LPEL had the greatest lactic acid concentration. A three-way interaction was observed ($P = 0.001$; Figure 1C) for acetic acid concentration and acetic acid was generally greatest for LO-LB and LO-CON. A three-way interaction was observed for butyric acid concentration ($P = 0.01$), but concentrations were low ($< 0.1\%$ DM) regardless of treatments. An interaction between microbial inoculation and storage length was observed for ethanol concentration and at 90 d of storage LB (0.5% DM) had the greatest ethanol concentration, followed by CON (0.4% DM), while LPEL (0.2% DM) was lowest. A three-way interaction was observed ($P = 0.001$; Figure 1D) for 1,2-propanediol concentration, where LB treatments generally had greater concentrations, although by 90 d of storage LO-CON did not differ from LB treatments. An interaction between microbial inoculation and pick-up height was observed for DM losses ($P = 0.03$), where at 90 d of storage LPEL (5.0% DM) had lower losses than CON and LB (8.6% DM, on average). An interaction between pick-up height and storage length was observed for ammonia-N concentration ($P = 0.001$). No differences in ammonia-N concentration were observed at 7 or 14 d. However, HI (6.3% CP) silage had a greater ammonia concentration than LO (5.2% CP) silage at 28 d of storage but had a lower ammonia concentration than LO silage at 90 d of storage (6.6 vs 8.4% CP, respectively). Dry matter concentration was 8 percentage-units greater ($P = 0.001$) and ash concentration 2.4 percentage-units greater ($P = 0.001$) for HI pick-up height than LO. Additionally, CP was 3.6 percentage-units lower ($P = 0.001$) for HI pick-up height than LO.

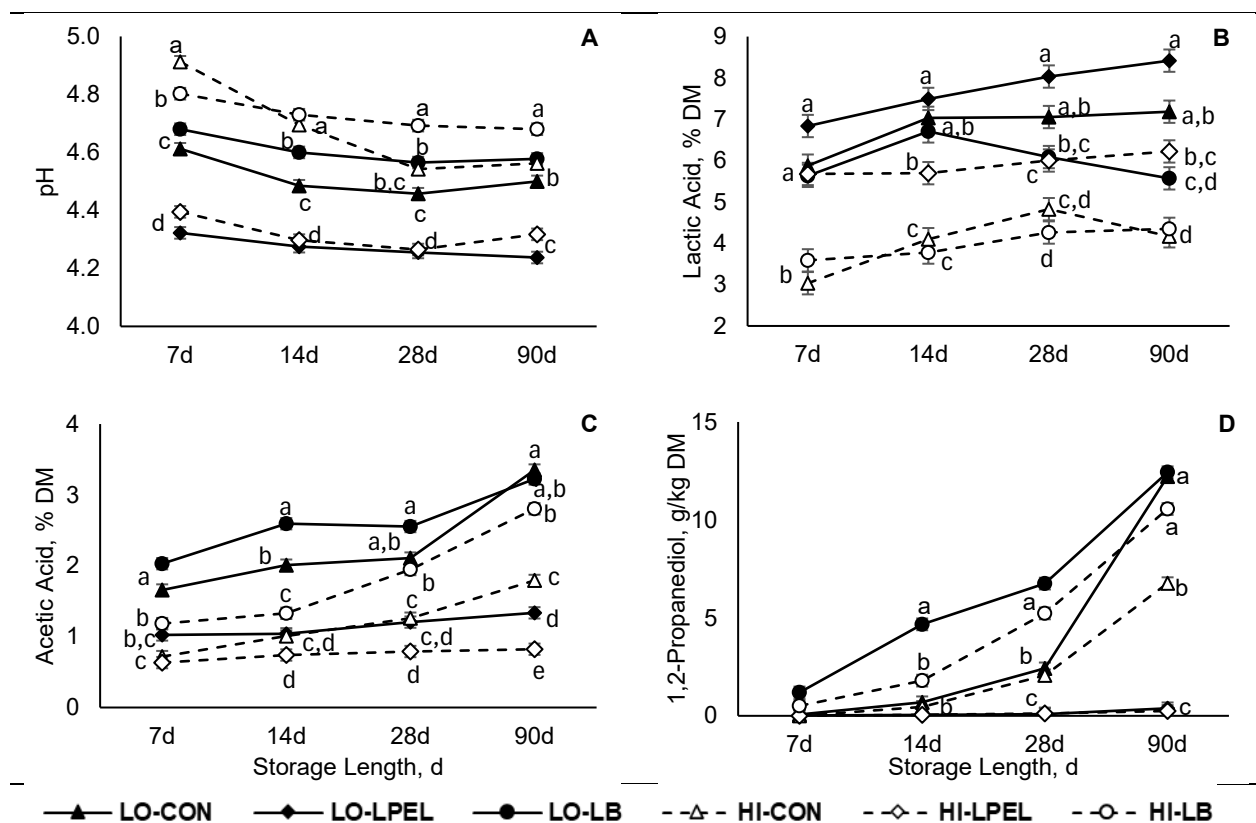


Figure 1. The effect of pick-up height during merging, microbial inoculation, and storage length on pH, lactic acid, acetic acid, and 1,2-propanediol of alfalfa silage. Treatments consist of pick-up height: LO (0 cm) or HI (6 cm); microbial inoculation: CON (distilled water), LPEL (*Lactiplantibacillus plantarum*, *Lactococcus lactis*, and *Enterococcus lactis*; 150,000 CFU/g forage), or LB (*Lactococcus lactis* and *Lentilactobacillus buchneri*; 150,000 CFU/g forage); storage length: 7, 14, 28 or 90 d. ^{a,b,c,d,e} Different superscripts denote differences among treatments within a storage length. (A) pH, $P = 0.001$; (B) Lactic acid (% DM), $P = 0.05$; (C) Acetic acid (% DM), $P = 0.001$; (D) 1,2-Propanediol (% DM), $P = 0.001$.

Conclusions

Increasing pick-up height increased DM but decreased CP, ash, and ammonia concentrations. Using a homofermentative microbial inoculant improved alfalfa silage fermentation and DM losses, while greater pick-up height during merging decreased ash concentration and nutritive value.

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Can a Biological Silage Inoculant Preserve High-moisture Hay?

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Keywords: high-moisture hay, lactic acid bacteria, organic acid salts

Introduction

Successful hay production relies on dry weather to achieve a target moisture content below 15%, which helps minimize the risk of fire (Martinson, 1993). The return on investment for using propionic acid-based additives on large round bales to enhance the nutritive value of high-moisture hay has been shown to be unfavorable (Coblentz and Bertram, 2012). Certain strains of lactic acid bacteria possess the ability to utilize oxygen during the initial aerobic phase of ensiling (Hindrichsen et al., 2012). Applying these strains in combination with heterofermentative lactic acid bacteria to high-moisture hay can generate anaerobic conditions in the core of the bale, promoting anaerobic fermentation. Meanwhile, the surface of the bales can naturally dry during storage. The objective of the study was to investigate the effects of a commercial silage inoculant containing a combination of oxygen-scavenging lactic acid bacteria (LAB) *Lactococcus lactis* and heterofermentative *Lentilactobacillus buchneri*, and a commercial chemical feed additive separately on the fermentation and hygienic quality of high-moisture grass hay prepared in large round bales.

Materials and methods

The grass was harvested at the flowering stage and wilted to approximately 72 % dry matter (DM). It was chopped to a theoretical particle length of 4.5 cm and harvested into round big bales with a diameter of 1.2 m, using a round baler set to the highest possible pressure. Five bales were prepared for each treatment group. The control bales (CON) remained untreated, while the inoculated bales (FC) were treated with SiloSolve® FC (Novonosis, Lyngby, Denmark) in a dosage of 200,000 total colony-forming units (CFU) per gram of forage including *L. buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037). The CHEM bales were treated with a chemical silage additive, consisting of a mixture of 259 g/l sodium benzoate and 94 g/l sodium propionate, applied at a dosage of 4.1 liters per ton of forage. The bales were stored on their flat ends on wooden pallets in a well-ventilated hall, without being wrapped in plastic. A data logger (Haytech) was inserted into the center of the top of each bale for temperature monitoring. After 102 days of aerobic storage, the bales were weighed, inspected visually for spoilage, and then cut into upper and lower halves using a silo block cutter. Samples were collected from both the core area and outer layers to assess nutritional value, fermentation parameters, and hygienic quality, including counts of lactic acid bacteria, Clostridia, aerobic bacteria, yeast and mold. Data were analyzed using the PROC MIXED procedure in SAS, with treatment as the main fixed effect. Statistical significance was defined at $P < 0.05$.

Results

Fresh herbage had a DM content of 67.7 to 77.1 %. It contained high-level water-soluble carbohydrates (WSC, 207 g/kg DM). During storage, microbial activity led to a significant rise in bale temperature, with recorded peaks reaching up to 60 °C. Most bales attained their maximum temperature within the first two days. This was followed by a slight cooling period before temperatures rose sharply again. Cooling occurred slowly and was not completed by the end of the trial. The moist hay in the bales dried out throughout the storage period, as indicated by the DM content (Table 1). The pH value of the fresh forage was 6.1. Minimal fermentation activity was observed during storage, as evidenced by the pH and acidity levels. After storage, the pH in the core areas was lower compared to the outer layer, with no differences between treatments. The synthesis of acids was very weak across all variants and sampling zones, with the highest concentration of lactic acid of 4.0 g/kg DM found in the core area of the CON. No acetic acid was detected in the core of the FC bales, indicating that *L. buchneri* did not perform effectively under these conditions. The low numbers of LAB, with no differences between treatments, suggest that the additives did not affect microbial activity during storage. It indicates that, on the one hand, the moisture content was too low to allow the growth and metabolism of applied bacteria. On the other hand, the anaerobic conditions were not established in the core of the bales.

Yeasts were under the detection limit in all bales. Molds were dominated by *Aspergillus* species detected in the core and outer layer in all bales and reached numbers up to 8 Log₁₀ CFU/g. According to the VDLUFA (2024), hay containing molds of *Aspergillus* species exceeding 6 Log₁₀ CFU/g is classified as excessively spoiled and unsuitable for animal feed.

Table 1. Characteristics of high-moisture bales (n=5 per treatment) prepared without inoculation (CON) or treated with SiloSolve® FC (FC), and chemical additive (CHEM). Values in brackets are from less than 3 repetitions.

Treatment		CON		FC		CHEM	
		core	outer layer	core	outer layer	core	outer layer
Dry matter	%	83.3	82.9	83.4	83.1	84.1	83.1
pH		5.45 ^a	7.21 ^b	5.59 ^a	7.16 ^b	5.79 ^{ab}	6.68 ^{ab}
Lactic acid	g/kg DM	4.0 ^a	2.4 ^a	3.0 ^{ab}	2.0 ^b	2.7 ^{ab}	2.1 ^b
Acetic acid	g/kg DM	(0.4)	0.8 ^a	n.d.	0.7 ^a	n.d.	0.6 ^a
LAB	Log ₁₀ CFU/g	1.8	1.9	2.4	1.3	1.9	1.3
MG2	Log ₁₀ CFU/g	3.8	5.7	3.9	5.6	4.3	6.0
MG4	Log ₁₀ CFU/g	3.4	4.0	(3.4)	(7.1)	(3.5)	(7.4)
MG5	Log ₁₀ CFU/g	6.6	7.3	6.4	7.2	6.7	7.2
MG6	Log ₁₀ CFU/g	n.d.	(4.3)	n.d.	3.7	n.d.	3.3

LAB = lactic acid bacteria; MG= microbial groups according to VDLUFA (2024) orientation values for the assessment of microbiological quality of feedstuffs, MG 2 = spoilage indicating bacteria: *Bacillus* spp., *Micrococcus* spp., *Staphylococcus* spp., MG4 = *Fusarium* spp., *Acremonium* spp., MG5 = *Aspergillus* spp., *Wallemia* spp., MG6: *Mucorales* spp.

Values in rows with different superscript letters are different at $P < 0.05$ (Tukey-Kramer-Adjustment)

n.d. – not detectable

Conclusions

Neither a bacterial inoculant nor a chemical additive effectively prevented spoilage in hay bales, with moisture contents ranging from 23 % to 32 %. While minimal fermentation occurred in the core areas, it was insufficient to inhibit the activity of undesirable microorganisms, resulting in compromised bale quality. For high-moisture hay, wrapping bales in plastic may offer a more effective preservation method when wilting to moisture levels below 15 % is not achievable.

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Improving the Quality of Haylage for Horses by Adding a Biological Silage Additive

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Keywords: haylage, extensive grassland, fructan, hygienic quality.

Introduction

In Northern Germany, the growths of extensively used grassland are often first harvested when the grasses have already finished flowering. Then the grass has a high fiber content and usually has an increased epiphytic population of yeasts and molds. Preserved as hay or haylage, it is preferably used to feed extensively kept cattle, horses or sheep. Horse owners, in particular, prefer very dry haylage. However, the hygienic quality is often reduced as no proper fermentation takes place in the higher dry matter (DM) range. The further problem is the low stability under the influence of air during feeding.

It is also crucial for horse feeding that the content of water soluble carbohydrates (WSC) especially fructans is low, as these compounds can trigger laminitis (van Eps & Pollit, 2008).

Therefore, a three-year project (2021 – 2023) investigated whether adding a biological silage inoculant could improve the quality of the haylage.

Materials and methods

An extensively managed grassland (about 50 % *Holcus lanatus* and *Alopecurus pratensis*) with a high proportion (23 %) of herbs was mown in mid-June. Swath samples (n=3) served to test the wilted grass for feed value parameters, especially WSC and fructans, hygiene and counts of lactobacteria (LAB). Round bales were produced with a standard bale-wrapper with high pressure but without knives after one or two days of lying in the field. To avoid cross-contamination, the first five bales were untreated and used as a control (CON). The next five bales were treated with a biological silage additive (SA) containing *Lactococcus lactis* DSM11037 and *Lentilactobacillus buchneri* DSM 22501 as recommended by the manufacturer. After wrapping with eight layers of stretch film, bales were instantly taken to the farm, weighed and stored on plastic pallets.

Bales were weighed again before feeding to estimate the DM loss caused by ensiling. During winter, bales were opened successively according to the feed requirements on the farm. Samples were taken and analyzed for DM, WSC, pH, fermentation acids, alcohols, molds and yeasts as well as for aerobic stability according to Honig (1990). After the aerobic stability test (ASTA), the haylage was checked for visible signs of aerobic spoilage and the pH was measured again. Statistical analyses were done by SPSS 23[®] using Kruskal-Wallis-test to identify significant differences ($P \leq 0.05$) between the treatments, separately for each year.

Results and discussion

The grass was wilted to a DM content of 573 g/kg in 2021, 739 g/kg in 2022 and 575 g/kg in 2023 (Table 1). Ash content was lower than 65 g/kg DM. In accordance with the late stage of maturity of the grasses, the protein content was on average 65 g/kg and the fiber content was high (crude fibre 307 g/kg, aNDFom 613 g/kg DM, ADFom 349 g/kg DM). Nevertheless, the grass contained sufficient WSC (140 to 190 g/kg DM), with high proportion of fructans (38 to 78 g/kg DM). The epiphytic population of LAB was low in all three years ($3.7 \log_{10}$ cfu/g), whereas molds and yeasts were detected in larger numbers (5.2 and $5.6 \log_{10}$ cfu/g).

Bales weights differed between 383 kg in 2022 and 510 kg in 2021. Usage of the additive showed reduced DM losses in all three years, but without significance to control within each year. In 2022, DM losses were the lowest (≤ 0.9 %) due to the minimal intensity of fermentation in the dry material. The reduced activity of LAB was reflected in the slight reduction in the pH value and the low levels of lactic acid and acetic acid. Although the WSC content has not been reduced as much, the fructan content has decreased. This matches the results of Merry et al. (1995), who reported a reduction in fructan content in grass at a pH value below 6. With one exception because of damaged plastic film, the hygienic quality was high. The highest number of molds and yeasts were $3.8 \log_{10}$ cfu/g and $4.5 \log_{10}$ cfu/g with no detection in 70 % of the samples or 50 % respectively. The dry haylages did not show any rise in temperature during aerobic storage, but a rise in pH, which indicated spoilage. In addition deterioration by molds was visible in some cases. SA treatment did not lead to any impact on dry haylage quality.

In the years 2021 and 2023, treatment with SA protected against faulty fermentation caused by clostridia and yeasts, leading to lower pH values as well as to a higher content of acetic acid and 1,2-propanediol. The significantly higher ethanol content in the untreated haylage indicates increased yeast activity during fermentation, which led to a greater reduction in the WSC content, particularly in 2021. The WSC and fructan content were significantly reduced compared to the original material with and without inoculation.

Three of the moist haylage bales had minor damage to the plastic film and therefore showed higher amount of germs. Apart from these exceptions, the hygienic quality was very good with maximum mold and yeast counts of $3.3 \log_{10}$ cfu/g and $4.7 \log_{10}$ cfu/g. No molds were detectable in 75 % of the samples and no yeasts in 50 %. In the 2021 ASTA, reheating and microbial deterioration were observed in the untreated silages after an average of 8 days,

which led to an increase in the pH value. The SA treated haylages did not show any signs of aerobic spoilage. In contrast, no temperature rise was detected in 2023 ASTA. However, CON haylages showed again an increase in pH value and visible spoilage.

No warming or visible spoilage could be detected during the mostly five-day feeding of the haylage bales on the horse farm. The horses seem to like eating the feed.

Table 1. Effects of ensiling without (CON, n=5) or with inoculation (SA, n=5) on haylage quality compared to the fresh grass (n=3). Comparisons between means were carried out using Kruskal-Wallis-test separately for the individual years. Means within a column not followed by the same letter are different ($P \leq 0.05$).

Year	Variant	DM [g/kg]	Sugar	Fructan	WSC [g/kg DM]	LA	AA	BA	E	1.2P	DM loss [%]	pH	pH _{out}
2021	Fresh	573 ^a	113 ^a	63 ^a	176 ^a								
	CON	502 ^b	60 ^b	18 ^b	79 ^b	19	5 ^a	2.8	21 ^a	0.5 ^a	2.0	5.4 ^a	6.5 ^a
	SA	533 ^{ab}	49 ^b	19 ^b	68 ^b	20	14 ^b	0.4	6 ^b	5.8 ^b	1.4	4.7 ^b	4.6 ^b
2022	Fresh	739	112	78 ^a	190 ^a								
	CON	718	82	42 ^b	125 ^b	4	0.6 ^a	n.d.	8	n.d.	0.9	5.7	6.0
	SA	725	100	41 ^b	141 ^{ab}	4	1 ^b	n.d.	5	n.d.	0.6	5.6	6.2
2023	Fresh	575	102	38 ^a	140 ^a								
	CON	559	66	14 ^b	80 ^b	12	3 ^a	n.d.	19 ^a	n.d.	1.3	5.3 ^a	8.1 ^a
	SA	566	86	17 ^c	103 ^b	20	11 ^b	n.d.	7 ^b	3.3	0.6	4.7 ^b	5.2 ^b

DM: dry matter, WSC: water soluble carbohydrates, LA: lactic acid, AA: acetic acid, BA: butyric acid, E: ethanol, 1.2P: 1.2-propanediol, pH_{out}: pH after aerobic stability test, n.d.: not detectable

Conclusions

Grass from extensively managed areas may have too high levels of WSC and fructan for use as horse feed. In the trial, preservation as haylage led to a reduction. However, to maintain consistently high quality and hygiene from bale opening to feeding, it is essential not to over-wilt the grass, ensuring that lactic acid fermentation can still occur. When the forage was wilted to a dry matter content of up to 575 g/kg, the application of a biological silage additive significantly improved fermentation quality. The treatment effectively inhibited undesirable microbial activity, including clostridia and yeasts, and enhanced aerobic stability. In contrast, when the grass was more heavily wilted, the application of the inoculant did not result in quality improvements.

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Forage Mixture and Ensiling Period Effect on the Chemical Composition of Unsalable *Amaranth-Megathyrsus* Silage

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Keywords Forage, Silage, Silo

Introduction

The dependent of smallholder ruminant farmers on natural pasture as source of feed for their animals is a norms especially in Nigeria (Lawal-Adebawale, 2012) and the excesses of these fodder during the rainy season can be conserved in form of silage. *Megathyrsus maximus* has been reported as one of the prominent natural grass species that is readily available as feed for ruminants in the tropics (Dele, 2012). In Nigeria, 60% of perishables fruits and vegetables are loss after harvesting (Ogundele, 2022) and the disposal of these products have become the source of pollution to the environment (Adejumo and Adebiyi, 2021) but could be channeled into livestock production. The aim of this study is to determine the chemical composition of *M. maximus* and unsalable *Amaranthus hybridus* silage as affected by ensiling period.

Materials and Methods

Megathyrsus maximus was harvested from the natural pasture at 8 weeks of regrowth while the unsalable *Amaranthus hybridus* was sourced from fruits and vegetables markets in Abeokuta, Nigeria. They were chopped to 2 – 3 cm, wilted and compactly packed in a laboratory silo of 960 ml capacity. Four (4) forage mixtures; 100% *M. maximus* (M100), 90% *M. maximus* + 10% unsalable *A. hybridus* (M90+V10), 80% *M. maximus* + 20% unsalable *A. hybridus* (M80+V20) and 60% *M. maximus* + 40% unsalable *A. hybridus* (M60+V40) and with three (3) ensiling periods (3, 6, and 9 week) and replicated five (5) times. At the end of each ensiling period, silos were opened and samples were oven dried at 55°C until a constant weight was attained and milled. Crude protein (Kjeldhal, N×6.25) was determined according to AOAC (1995) procedure. Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were determined following Van Soest *et al.* (1991) procedure. The sodium and potassium content was determined using a flame photometer while calcium, magnesium and phosphorus using flaming in Atomic Absorption Spectrophotometer according to AOAC (1995) by wet ashing. Data were analyzed using General Linear Model (GLM) procedure in SAS 9.4. At 5% level of significance, significant differences among treatments were determined using Tukey Honest Significant.

Results

There was no significant ($P > 0.0001$) difference in the dry matter content of the silage as affected by ensiling period and forage mixture interaction. The content of CP, NDF, ADF, and Acid detergent lignin, were significantly ($P > 0.0001$) different and ranged from 10.47 – 13.17 %, 49.96 - 68.48 %, 41.05 – 48.83 %, 3.14 – 5.40 % respectively. Percentage NDF, ADF and ADL were peaked at 9 week ensiling period of M100 and least at 6 week of ensiling period of M60 + V40. Highest content of Ca, K and Mg was observed at 6 week of ensiling period of M60 + V20. Phosphorus content of the silage was affected ($P < 0.0001$) by the interaction effect of forage mixture and ensiling period.

Table 1. Effects of ensiling period on chemical composition of unsalable Amaranth-Megathyrus silage

Forage Mixture	Ensiling Period	DM	CP	NDF	ADF	ADL	Ca	P	K	Mg
		% DM					gkg ⁻¹ DM			
M100	3	91.12	10.95 ^{de}	65.67 ^b	48.04 ^a	5.01 ^{ab}	3.00 ^h	2.74 ^d	12.67 ^h	4.94 ^g
	6	91.34	10.69 ^{ef}	68.15 ^a	43.59 ^{efd}	4.48 ^{bc}	4.08 ^g	1.88 ^h	14.05 ^f	4.26 ⁱ
	9	90.92	10.47 ^f	68.48 ^a	48.83 ^a	5.40 ^a	3.84 ^g	1.75 ⁱ	12.89 ^h	4.71 ^h
M90+ V10	3	90.32	11.83 ^c	62.30 ^c	46.30 ^b	4.28 ^{cd}	5.76 ^f	3.26 ^c	15.73 ^f	6.63 ^d
	6	91.27	11.32 ^d	60.65 ^{cd}	44.82 ^{bcd}	4.59 ^{bc}	6.05 ^e	2.32 ^f	17.60 ^d	5.84 ^f
	9	89.60	12.11 ^{bc}	58.32 ^e	44.27 ^{cde}	3.62 ^{def}	8.76 ^b	2.25 ^f	17.06 ^e	7.46 ^{bc}
M80 + V20	3	90.90	12.93 ^a	60.49 ^d	42.19 ^{gh}	3.14 ^f	6.47 ^d	3.71 ^a	17.57 ^d	6.26 ^e
	6	90.43	13.15 ^a	57.55 ^e	43.15 ^{efg}	4.06 ^{cde}	7.64 ^c	2.55 ^e	18.19 ^c	6.73 ^d
	9	90.41	11.95 ^{bc}	56.93 ^e	44.14 ^{cde}	4.73 ^{abc}	7.45 ^c	2.15 ^g	17.06 ^e	6.69 ^d
M60 + V40	3	90.02	12.38 ^b	56.63 ^e	45.38 ^{bc}	4.23 ^{cd}	8.84 ^b	3.39 ^b	15.63 ^f	7.36 ^c
	6	89.78	13.17 ^a	49.96 ^f	41.05 ^h	3.37 ^{ef}	10.77 ^a	1.76 ⁱ	20.49 ^a	7.82 ^a
	9	90.47	12.00 ^{bc}	51.31 ^f	41.63 ^{gh}	4.05 ^{cde}	11.00 ^a	1.25 ^j	19.34 ^b	7.64 ^{ab}
SEM		0.29	0.12	0.75	0.33	0.10	0.33	0.09	0.31	0.15
P-Value										
FM		0.7069	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
EP		0.8975	0.0002	<0.0001	<0.0001	0.1069	<0.0001	<0.0001	<0.0001	<0.0001
FM x EP		0.9890	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

a,b,c,d,e,f,g,h,i,j : Means in the same column with different superscripts were significant ($P < 0.05$) different. DM = Dry Matter, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, ADL = Acid Detergent Lignin. Ca = Calcium, P = Phosphorus, K = Potassium, Mg = Magnesium, M = *Megathyrus maximus*, V = *Amaranthus hybridus*, FM = Forage mixture, EP = Ensiling period

Conclusion

Megathyrus maximus and unsalable *Amaranthus hybridus* silage at all mixture levels and ensiling periods produced silage with exceptional nutritional quality. However, the environmental hazard caused by unsalable vegetables especially *Amaranthus hybridus* can be reduced when ensiled with *Megathyrus maximus* and this would be of great economical value for farmers as it would improve the quality of their animals when fed quality silage and also, reduce the emission of green-house gas which gives us safer environment.

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Methane Index for Grass Silages: A Standardized Tool for Benchmarking Methane Emissions Globally

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Keywords: Methane Emissions, Forage, Grass silages, Climate change

Introduction

Methane is a significant environmental concern in agriculture worldwide, contributing to climate change. Governments globally are addressing this issue; for instance, in 2022, the Dutch Government introduced a National Methane Strategy to reduce methane emissions by 30% by 2030 compared to 2020 levels.¹ The agricultural sector, responsible for a large share of methane (CH₄) emissions, up to 78%.² Measures like feed additives, optimized livestock diets, and advancements in breeding are being implemented to create a more sustainable sector.

This study observed significant differences in methane emissions between years and seasons, prompting the creation of a Methane Index by Eurofins Agro. This Index benchmarks methane emissions in grass silages worldwide. This innovative tool supports promotes environmental sustainability in agriculture.

Materials and methods

Based on research conducted by Wageningen University, two unpublished models developed by Sebek et al., based on chemical parameters of silages, were utilized to predict potential methane emissions from grass silages. These models were applied to calculate the potential methane emissions for 47,422 grass silages harvested across Europe in 2022 and 2023. Additionally, to examine yearly fluctuations in methane excretion, we analyzed a dataset involving 347,185 production samples from grass silages harvested between 2015 and 2024 in the Netherlands.

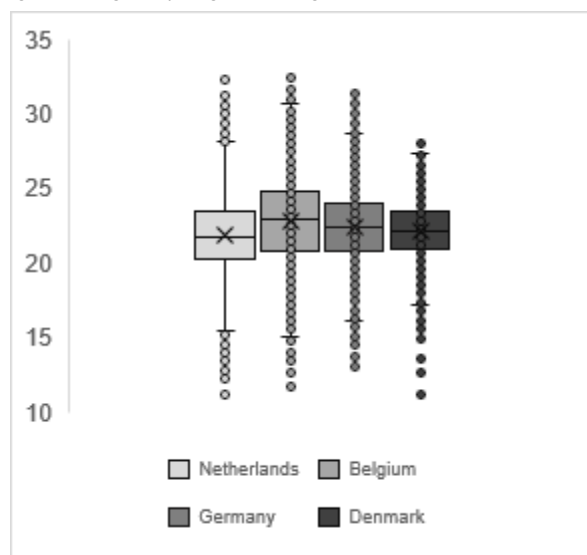
In the analysis of the production samples submitted to the Eurofins Agro laboratory in the Netherlands, the ADF model was preferred; however, when ADF was not available, the Crude Fiber model was used. The results were processed using Excel, and statistical methods, including regression analysis, were employed to ensure accuracy and consistency.

Results

The analysis revealed significant differences in methane emissions across various dimensions, including countries, seasons, and years. These findings underscore the complexity of methane emissions and the need for a comprehensive approach to benchmarking and reduction strategies.

The analysis of average potential methane emissions across the Netherlands, Belgium, Germany, and Denmark reveals significant variations, as shown in Figure 1. The Netherlands exhibits the lowest average emissions, while Belgium shows the highest. Additionally, Belgium's interquartile range (IQR) is the largest, indicating greater variability in methane emissions. Denmark has the lowest IQR, suggesting that methane emissions are more consistent.

Figure 1. Potential methane emissions (in gr CH₄/kg DM) in grass silages in the Netherlands, Belgium, Germany and Denmark.



The seasonal variation in potential methane excretion was analyzed, revealing distinct patterns throughout the year. As illustrated in Figure 2, methane excretion peaks during the spring season and reaches its lowest levels in autumn. This seasonal trend suggests that environmental or biological factors influencing methane production are more active in spring compared to autumn.

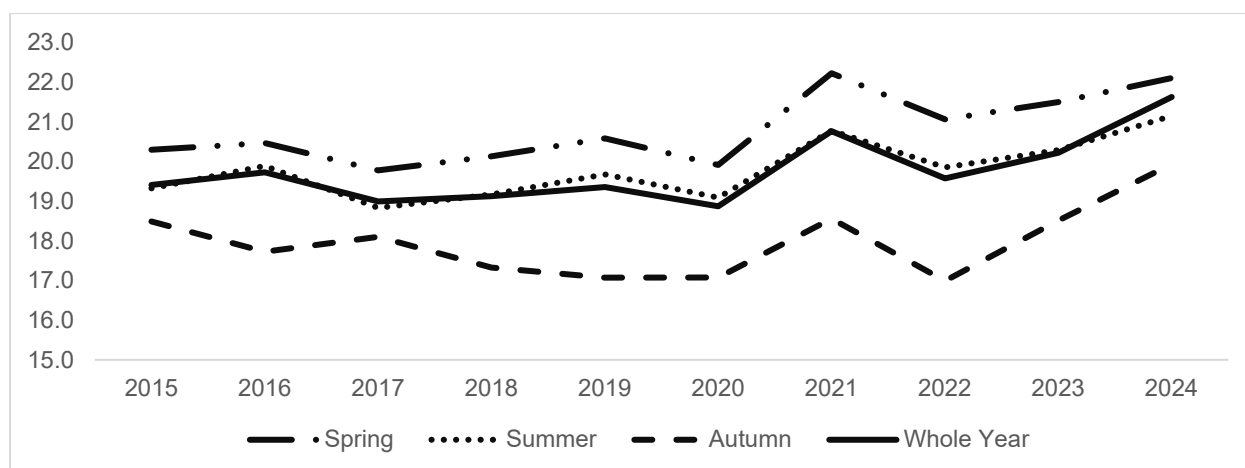


Figure 2. Seasonal Variation of Potential Methane (in gr CH₄/kg DM) Emissions in the Netherlands (2015-2024)

During the analysis, it became evident that benchmarking methane emissions based on country averages was not feasible due to the varying number of samples from different countries. To address this, an index was created to correct for seasonality and yearly fluctuations. This index allows for a more accurate comparison of methane emissions across different regions and time periods. The index evaluates whether the potential methane emissions of the silage, based on the Wageningen University models, are high or low.

Conclusions

The analysis of potential methane emissions across different seasons and countries revealed significant variations. The Netherlands exhibited the lowest average emissions, while Belgium showed the highest and most variable emissions. Denmark had the most consistent emissions profile. Additionally, the seasonal analysis indicated that methane emissions peaked in spring and were lowest in autumn.

The proprietary index, developed during the results analysis, proved essential for benchmarking methane emissions globally. This index corrects for seasonality and yearly fluctuations, enabling farmers to understand that high absolute methane emissions may be due to environmental conditions rather than poor management practices. More research on a wider scale is needed to identify effective management practices that can lower methane emissions and, consequently, reduce the methane index.

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Updating the Milk Index for Corn Silage: Milk2024 and Previous Versions

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Keywords: MILK2024, MILK2006, energy

Background

Corn silage typically makes up a large part of the diets of dairy cows, since it provides energy to support milk production and physically effective fiber to support rumen health. However, the nutritive value of corn silage ranges widely and with many variables to consider, evaluating the quality and value of corn silage can be difficult for producers. The MILK index was first created in 1990 to combine important nutrients into a single value that allows producers to compare the energy density (milk/ton) or energy yield (milk/acre) of forages. The MILK90 estimated TDN based on ADF concentration and intake based on NDF concentration, which were used together to estimate forage energy potential (Undersander et al., 1993). As research advanced, the MILK index was updated in 1995 to include dry matter digestibility (DMD) as an input, while ADF was removed. The MILK index was updated again in 2000 and adapted to use the same summative equations used in NRC (2001), but included a starch fraction (Shaver, 2006). The MILK2000 also replaced DMD with NDF digestibility (NDFD) as an input (Shaver, 2006). In 2006, a new version of the MILK index was introduced, that included variable estimates of starch digestibility (starchD) such as in vitro or in situ 7h starchD, a starchD regression based on DM concentration, kernel processing score, and the degree of starch access analysis (Shaver, 2006). The MILK2006 also still included options to add NDFD and was based on equations similar to those used in MILK2000 (Shaver, 2006). The MILK2006 quickly gained popularity and was widely used throughout the U.S. and the world to compare corn hybrids or silage and as a teaching and extension tool until 2024, when the MILK index was updated again. The goal of this review is to focus on the changes made in MILK2024 and highlight comparisons with previous versions of the MILK index.

Discussion

The MILK2024 spreadsheet uses equations based on NASEM (2021), with some modifications described in Diepersloot et al. (2024a). Notably, MILK2024 no longer uses calculations based on TDN, instead estimating DE directly, and requires a basal diet. Additionally, MILK2024 uses mechanistic models to estimate total tract digestibility of starch and NDF (using 7h starchD and 30 or 48 h NDFD) instead of using in vitro or in situ estimates of digestibility directly, as previous versions of the MILK index did. The updated digestibility calculations also slightly changed the nutrient inputs used in MILK2024, as undigested NDF (uNDF) concentration and 7h starchD (not required, but an option available in MILK2006) are now both required, while DM concentration was removed in MILK2024 but was required in previous versions. While MILK2006 and previous versions of the MILK index predicted the yield of 3.5% fat-corrected milk, MILK2024 predicts the yield of milk with 4.0% fat, 3.1% true protein, and 4.8% lactose. The MILK2024 model was compared with MILK2000 and MILK2006 using a large dataset of corn silage samples analyzed at commercial laboratories as shown in Table 1. Overall, MILK2024 predictions were lower, or similar depending on how MILK2006 is calculated, compared to MILK2006 and MILK2000. This can be explained by the greater components used to calculate milk yield in MILK2024 compared to other versions. Additionally, both the range and standard deviation of MILK2024 were lower compared to MILK2000 and MILK2006, suggesting that MILK2024 is less variable. Thus, smaller differences should be considered significant when using MILK2024 compared to previous versions. Additional comparisons between MILK indexes were explored by Diepersloot et al. (2024b) using the same dataset, which was ranked by different nutrients (such that all nutrients differed each time nutrients were ranked) and the overlap between the top and bottom 10% of samples were calculated. This exercise demonstrates the ability of MILK indexes to separate samples with different nutrient compositions. The overlap between the top and bottom 10% of samples ranked by NDF, NDFD, and starch was lower for MILK2024 than MILK2006, although MILK2000 was similar or slightly better than the MILK2024 for some, but not all nutrients. However, when the dataset was ranked by starchD, MILK2024 had a greater overlap between the top and bottom 10% of samples compared to MILK2006 calculated with 7h starchD instead of DM concentration or other options. Since MILK2000 and MILK2006 calculated with DM instead of 7h starchD do not include 7h starchD in any calculations, they were expected to have greater overlap between the top and bottom 10% of samples. However, MILK2024 was expected to outperform previous models due to the updated starchD calculations.

Table 1. The nutrient composition and predicted milk yield of corn silage samples obtained from the normal range (average $\pm$ SD; n = 101,025) of datasets from three commercial labs.

Item ¹	Average	SD	Minimum	Maximum
DM, % as fed	36.8	3.72	23.3	57.6
CP, % DM	7.6	0.51	6.6	8.8
NDF, % DM	38.4	2.30	32.4	47.7
NDFom, % DM	37.3	2.32	32.3	43.1
30h NDFD, % NDF	57.7	2.88	52.0	65.6
30h NDFDom, % NDFom	58.5	2.97	52.5	66.7
240h uNDFom, % DM	10.4	1.56	6.8	15.1
Starch, % DM	34.8	2.94	26.3	40.7
7h StarchD, % starch	77.1	5.35	65.4	88.2
EE, % DM	2.9	0.37	2.1	3.9
Ash, % DM	4.0	0.65	2.7	5.6
2000 kg milk/Mg	1835	78	1508	2057
2006 kg milk/Mg ²	1734	78	1414	1977
2006 kg milk/Mg ³	1493	58	1256	1748
2024 kg milk/Mg	1547	42	1400	1680

¹CP: crude protein; NDF: neutral detergent fiber; NDFom: neutral detergent fiber corrected for residual ash; NDFD: NDF digestibility; NDFDom: NDF digestibility corrected for residual ash; uNDFom: undigested NDF corrected for residual ash; starchD: starch degradability; EE: ether extract.

²Calculated with DM concentration (% as fed), rather than 7h starchD (% starch) in MILK2006.

³Calculated with 7h starchD (% starch), rather than DM concentration (% as fed) in MILK2006.

Conclusions

Overall, updates in MILK2024 appear to improve the biological merit of the MILK index, by establishing a milk composition more representative of modern dairy cows, incorporating more recent research and equations into predicted milk yield, and using estimated total tract NDF and starch digestibility. As a result, the overall performance of MILK2024 improved compared to MILK2000 and MILK2006. However, MILK2024 appears less sensitive to 7h starchD than MILK2006 (when 7h starchD is used as an input), which means that future updates will need to explore additional options to predict total tract starchD as more research, models and assays become available.

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Publishing Silage Trials in the Journal of Dairy Science

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Keywords: experimental design, statistical power, representation, contextualization.

Silages made from whole plants (e.g., alfalfa, corn, sorghum, or triticale) and grains (e.g., high-moisture corn or barley) are key ingredients in dairy cattle diets. Silage-related research typically evaluates pre-harvest, harvest, or post-harvest factors affecting silage quality, although some other silage-related studies may address analytical procedures. Adesogan et al. (2020) raised concerns about the criteria for accepting or rejecting silage-related manuscripts in the *Journal of Dairy Science*. Although the Editor-in-Chief of the journal addressed these concerns directly (Kononoff, 2020), the organizing committee of the *XX International Silage Conference* requested further comment on guidance on best practices for conducting and preparing articles for publication. This abstract intends to identify common issues in silage-related studies and propose strategies for enhancing the quality of scientific publications.

One common mistake in silage-related publications is a flawed experimental design that fails to distinguish between experimental and observational units (Belo et al., 2016; Lamberson, 2016). This issue often arises in silage-related studies, particularly when inoculants or additives are added to forages to test their impact on dry matter recovery, fermentation characteristics, or nutritional composition. Some studies apply treatments to a single forage pile that is later fractionated and ensiled in mini-silos. In this case, measurements represent observational units but not experimental units (Belo et al., 2016; Lamberson, 2016). Other studies do not clarify whether the investigators independently applied treatments to replicated piles of forage before ensiling or to a single pile that is later fractionated and ensiled multiple times. To improve clarity the authors must provide sufficient detail on how they applied treatments to the experimental units. Unlike the previous cases, Schmidt and Kung (2010) and Romero et al. (2017) fractionated forage biomasses from n fields or plots (i.e., replicates) into p piles within each field or plot and then randomly and independently applied one of the t treatments to each pile, where p and t were equal. In the latter two cases, each pile is the experimental unit independently subjected to a treatment, therefore adhering to the three essential conditions for adequate statistical inference: replication, independence, and randomization (Cochran and Cox, 1992). The importance of these principles lies in the potential for erroneous conclusions when applying treatments to only one pile per treatment. If the piles differ in composition due to sampling variation, the lack of independent replication could lead to inaccurate or biased results, as the initial pile compositions vary.

Research should test technologies and innovations across different scenarios to ensure their effectiveness. In agronomical research, this often involves replicating studies over time, across locations, or both to assess how technologies or innovations perform in varying environments. While the *Journal of Dairy Science* does not mandate temporal or spatial replication (i.e., testing across different years or locations), studies must evaluate inoculants or additives using samples from diverse and independent sources. Testing technologies or innovations on a single source of alfalfa, for example, limits the findings to that single source of alfalfa. Testing technologies or innovations from various plots from a single field also limits the findings to that single field. Schmidt and Kung (2010) collected whole-plant corn samples and tested treatments on forages from five varieties grown at separate locations, ensuring experimental units as being independent and representative of the sample population.

Replicating the forages or grains and the treatments is essential to ensure adequate representation when evaluating pre-harvest and harvest factors. Ferreira et al. (2014), for example, assessed the effect of four corn planting populations (i.e., treatments) on forage quality and digestibility. To ensure some representation, the hypothesis was tested using two different corn varieties. Replication of forages or grains is also important for adequate statistical inference. For example, multiple varieties of each genotype are needed to test a hypothesis stating that the fiber digestibility is greater for brown midrib genotypes than for conventional genotypes. Similarly, multiple sources of each grain type are needed to test a hypothesis stating that starch digestibility is faster for oats grain than for corn grain. Ferreira et al. (2018) estimated the in situ ruminal starch digestibility for five varieties each of corn, hulled barley, and hull-less barley, and concluded that starch from both types of barley digested faster than starch from corn. However, the authors also found that certain corn varieties exhibited higher starch digestibility than some barley varieties, which highlights that using a single variety per grain type could have resulted in a different conclusion.

Performing a statistical power calculation is a good practice for adequate statistical inference. In this line, it is recommended that silage-related studies include a statistical power calculation to justify the number of experimental units used. In addition, including a statistical power analysis is an opportunity to contextualize and enhance the understanding of the aims and impacts of the study. For instance, assuming a statistical power of 0.80, a type I error probability (α) of 0.05, and a standard deviation (SD) of 0.025 for silage pH, a minimum of 4 experimental units would be required to detect a 0.2-unit difference in silage pH. In contrast, under the same power conditions but with an SD of 2.64% for dry matter recovery, 12 experimental units would be necessary to detect a 5%-unit difference in DM recovery. Beyond from reading the study objectives, reading a statistical power calculation can provide perspective to reviewers, editors, and readers.

In broad terms, the *Journal of Dairy Science* welcomes “scholarly work that relates to the production and processing of milk or milk products intended for human consumption” (Kononoff, 2020). In this regard, silage-related studies should

discuss the implications of the study to dairy production. In recent years, the *Journal of Dairy Science* has received numerous studies evaluating the effect of inoculants or additives on silage microbiome. Unfortunately, despite their sound experimental methods, several of these studies have not been accepted because they fail to contextualize the findings within the dairy industry. The latter also stands for plot studies. For example, some reviewers and editors may perceive plot studies (Ferreira et al., 2014) as agronomic studies rather than dairy-focused. Although it does not focus on agronomic studies, the *Journal of Dairy Science* welcomes plot studies aimed at generating samples that evaluate variables related to milk production (Kononoff, 2020). In many cases contextualizing the study need not be lengthy or exhaustive but should be included.

Content in published journals reflects the collective effort and knowledge of its authors, reviewers, and editors. Some published and historical studies may include experimental designs or methods that, given advances in knowledge, can be improved in newer publications. Consequently, while seeking excellence in research, the existence of historical and published methods should not be the sole justification for the design of new experiments.

To conclude, researchers focused on forage and silage research play a crucial role in the journal's community. Forage quality and management are pillars of sustainable dairy production, and the *Journal of Dairy Science* is committed to supporting and valuing research that strengthens these key areas. As such, proper manuscript preparation requires careful attention to experimental design, power analysis, statistical inference, comprehensive representation of treatments, and meaningful contextualization of findings.

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Comparison of Classical and Molecular Methods to Study the Microbiota Along the Silo Face on Farm Corn Silage

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Keywords: farm silage, metagenomic, microbial community, culture-dependent methods

Introduction

The microbial groups that develop during each phase of ensiling influence the end quality of the silage. Improving the understanding of silage microbial community dynamics is critical to develop strategies to ensure successful silage production. The identification of microbial communities of silages was traditionally performed using culture-dependent methods that mainly report a quantitative count of culturable microorganisms, but with a high proportion of microbes that remain uncultured. Through culture-independent techniques, it is now possible to characterize complex bacterial communities, that can be integrated with traditionally culture-dependent methods to have a more comprehensive quantitative approach (McAllister et al., 2018). The aim of this study was to analyze the uniformity of microbiota along the silo interface of corn silages of commercial dairy farms using both culture-dependent enumeration methods and culture-independent methods (i.e. 16S rDNA gene and ITS amplicon sequencing).

Materials and methods

The survey was carried out on five dairy farms located in North-West Italy during the summer. Samples were collected from three sampling areas of each corn silage bunker silo: the core (C), and 2 peripheral areas, close to the sealant film (A1), and the outermost 0.20 m of visibly spoiled silage, which is usually discarded by farmers (BA1). Silages were analyzed for microbiota, DM content, pH, fermentative profile, and aerobic stability. Microbial count was evaluated using culture-dependent methods (specific culture media for yeast and mold, lactic acid bacteria, enterobacteria, and acetic acid bacteria). Microbiota was determined by culture-independent method by extracting DNA from each sample and by amplifying the V3 and V4 regions of the 16S rRNA for bacteria and fungi, and the internal transcribed spacer (ITS)-1 region for fungi. Alpha diversity was assessed by Shannon diversity index.

Results

The studied farms showed different management of the bunker silos that influenced the silage temperatures and the surface of moldy areas of the silo interface (Table 1).

Table 1. Sealing strategies and silage temperatures over the silo interface of the studied bunker silos.

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Days of conservation	137	112	216	69	73
Temperature (°C)					
Air	27.7	28.4	34.7	36.4	29.6
C	23.1	24.3	21.2	20.0	20.8
BA1	33.9	31.7	39.1	29.0	28.2
A1	44.7	39.8	48.9	38.0	34.6
Surface visible moldy (%)	2.7	0.8	2.4	0.0	9.5
Feed out rate (m/d)	0.123	0.157	0.109	0.212	0.251
Plastic film on the wall	yes	no	no	yes	yes
Plastic film (< 100 µm)	no	no	no	yes	yes
Plastic film (> 100 µm)	yes	yes	yes	yes	yes
Weighting	Tires	Tires	Tires	Tires	Tires + bags
Inoculum treatment	None	<i>Lentilactobacillus buchneri</i>	None	<i>L. buchneri</i> + <i>L. plantarum</i>	None

The pH ranged from 3.51 to 3.92, 3.50 to 3.98, and 3.93 to 7.68 for C, BA1 and A1 areas, respectively. Lactic acid was detected in C and BA1 areas, whereas it was below detection limit in A1. Acetic and propionic acids were greater in A1 than in the other areas of the bunker. Butyric acid was detected only in A1 of some farms (Figure 1).

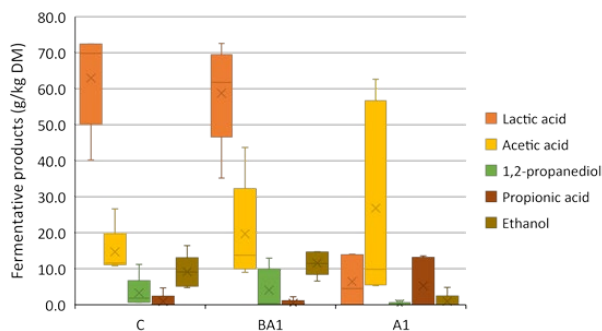


Figure 1. Main fermentative products of samples in different areas of bunker silos.

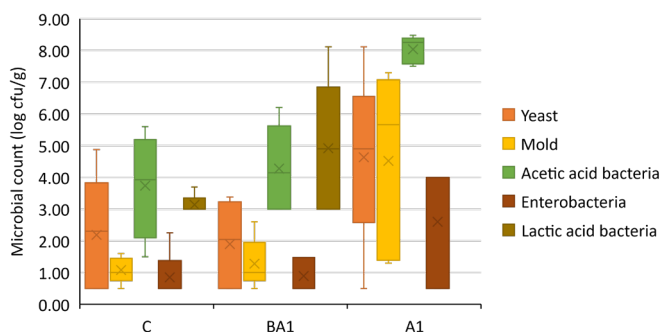


Figure 2. Microbial count of samples in different areas of bunker silos.

Yeast count was greater in A1 than in C (Figure 2). Enterobacteria and acetic acid bacteria count increased from C to A1 areas. The microbiota analysis showed *Firmicutes* dominated the C and BA1 samples (Figure 3), whereas *Bacteroides* and *Proteobacteria* the A1 samples. *Acetobacter* was the main genus detected in A1 samples (Figure 4) corresponding to acetic acid bacteria count greater than 8 log cfu/g. When lactic acid bacteria count was greater than 4 log cfu/g *Lactobacillus* genus was the main genus detected in the sample. *Kazachstania* was the main representative yeast genera, whereas the *Eurotiales* fungal order was the main detected, with *Aspergillus* genus dominated A1 samples. Alpha diversity was affected by farm and by sampling area.

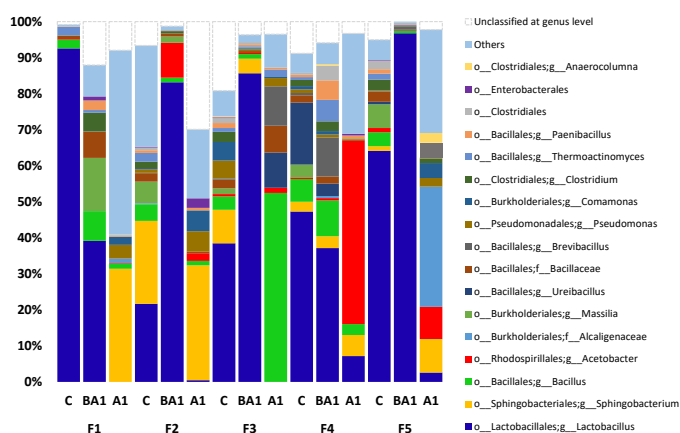


Figure 3. Taxonomic profile, relative abundance and count of the bacterial microbiome. Lactic acid bacteria were not counted in A1 section.

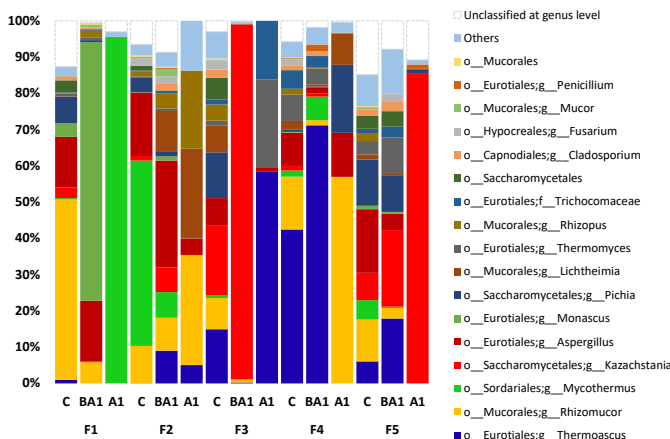


Figure 4. Taxonomic profile, relative abundance and count of the fungal microbiome.

Conclusions

The evaluation of microbial communities of silages allows to evaluate the qualitative microbial quality of silages, however this technique needs to be integrated with traditionally culture-dependent methods to have a quantitative approach. The bunker management strongly influenced the fermentative and microbial characteristics of silages, in particular for BA1 and A1 areas. Better microbial qualitative characterization will help farmers in managing bunker and preventing aerobic deterioration.

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Silage Fermentation and Aerobic Stability of Whole Crop Corn Ensiled in Laboratory or Bunker Silos as Affected by Lab Inoculum

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Keywords: aerobic stability, LAB inoculum, microbial quality, silo size

Introduction

Obligate heterofermentative lactic acid bacteria (LAB) inocula of *Lentilactobacillus buchneri* group have been used to improve aerobic stability of silages. Recently a combination of new strains of *L. hilgardii* and *L. buchneri* have been selected to improve the aerobic stability of silages. The effect of LAB inocula on the fermentation pattern and aerobic stability of silages was usually evaluated in laboratory silos of different sizes from few milliliters to several liters under controlled conditions. These standardized conditions could bias the results by misrepresenting the effects obtained on farm, where larger quantities of silage are stored outdoors under variable environmental conditions. The aim of this study was to assess the effect of LAB inoculum on the fermentation profile, microbial characteristics, and aerobic stability of whole corn crop ensiled in bunker silos or in laboratory silos.

Materials and methods

The crop was chopped with a precision forage harvester to a theoretical cut length of 10 mm at the $\frac{3}{4}$ milk line stage. The chopped corn was ensiled either in 20-L plastic laboratory silos and in farm bunker silos, as untreated (NT), or LAB treated (T) forage (Magniva Platinum 1, Lallemand SAS, France). The LAB inoculum was applied at the label recommended dose with the precision forage harvester treatment system. The fresh forages were sampled prior to ensiling. Laboratory silos were conserved at ambient temperature for 120 d. At the same time of laboratory silos opening, treated and untreated silages ($n = 4$) were collected from the bunker silo face. The herbage and silage samples were split into four subsamples. One sub-sample was analyzed immediately for the DM content. A second fresh sub-sample was used to determinate the pH. A third sub-sample was extracted in H_2SO_4 and used for quantification of the fermentation products by HPLC. The fourth subsample was used for the microbial analyses. Mold and yeast counts were determined in YGC agar, acetic acid bacteria following Spoelstra et al. (1988) with minor modifications, and the LAB were determined on MRS agar. DM losses were calculated for laboratory silos. Aerobic stability was determined under controlled conditions at 20°C and defined as the number of hours the silage remained stable before rising more than 2°C above ambient temperature. Data were analyzed separately for laboratory silos and bunker silos by unpaired t-test.

Results

The DM at harvest was 36% and the fresh matter presented microbial count typical of corn silage at harvest with yeast and mold around 7.0 log cfu/g (Table 1).

The main fermentative and microbial characteristics of treated and untreated silages are reported in Table 2. All the silages were well fermented with a dominance of lactic acid fermentation. For the conservation in laboratory silos the pH was higher in T than NT silages, due to lower amount of lactic acid, whereas acetic acid was higher in T than in NT both in bunker and laboratory silos. 1,2-propanediol was only detected in T silages with higher value in laboratory silos. The yeast count was lower in T silages in laboratory silos than in NT silages, whereas in bunker silo yeast and mold counts were under the detection limit. The LAB count of silages at 120 d was higher in T silages than in NT with higher values in laboratory silos than in bunker silos. DM losses of laboratory silos were typical of whole crop corn silage conserved for more than 100 days with an average value of 2.72%. The T silages showed higher aerobic stability both in laboratory silos and bunker silo. The increase in aerobic stability due to the treatment was +117 and +76 h in laboratory silos and bunker, respectively. The increase in acetic acid content can partially explain the increase in the aerobic stability of silages after 120 d of conservation. However, the magnitude of aerobic stability and yeast and mold count reduction were higher in bunker than in laboratory silos both for T and NT silages. A relationship between acetic acid content and aerobic stability was found both in laboratory silos ($y = 15.8x - 60.9$, $R^2 = 0.95$) and bunker silos ($y = 16.2x + 102.5$, $R^2 = 0.52$) with differences in the magnitude of relation. These results showed that there are some undetected factors which contribute to the increase of the aerobic stability of bunker silages, independent of the amount of acetic acid produced over conservation. Further investigations on factors affecting silage aerobic stability in bunkers silages should be performed, to aid farmers to better manage their silos.

Table 1. Chemical and microbial characteristics of the herbage prior to ensiling

Parameters*	T	NT	P-value	SEM
DM (%)	37.6	35.3	0.051	1.133
pH	5.82	5.74	0.047	0.039
Yeasts (log cfu/g)	6.85	7.13	0.011	0.143
Molds (log cfu/g)	6.90	7.44	0.045	0.266
Acetic acid bacteria (log cfu/g)	8.68	9.78	0.051	0.315
LAB (log cfu/g)	6.04	5.98	0.854	0.125

* cfu =colony forming unit; DM = dry matter; FM = fresh matter; LAB = lactic acid bacteria; SEM = standard error of the mean. T = treated with Magniva Platinum; NT = untreated. Values represent the mean of four replicates.

Table 2. Fermentative and microbial characteristics of treated and untreated silages

	Laboratory silo			Bunker silo		
	T	NT	P-value	T	NT	P-value
pH	3.77	3.65	0.005	3.43	3.41	0.385
Lactic acid (g/kg DM)	34.1	44.0	0.017	56.7	58.4	0.684
Acetic acid (g/kg DM)	15.1	7.2	0.005	10.4	6.4	0.003
1,2-propanediol (g/kg DM)	7.7	<0.1	-	3.7	<0.1	-
Ethanol (g/kg DM)	12.6	16.7	0.147	11.1	10.5	0.704
Yeast (log cfu/g)	2.76	4.87	0.003	<1.00	<1.00	-
Mold (log cfu/g)	<1.00	1.16	-	<1.00	<1.00	-
LAB (log cfu/g)	9.29	7.71	0.005	4.78	3.00	<0.001
Acetic acid bacteria (log cfu/g)	1.93	5.10	<0.001	1.50	2.57	0.013
Aerobic stability (h)	174	57	0.049	277	201	0.029
DM losses (%)	3.05	2.39	0.136	-	-	-

* cfu =colony forming unit; DM = dry matter; FM = fresh matter; LAB = lactic acid bacteria. T = treated with Magniva Platinum; NT = untreated.

Conclusions

Even if the LAB inoculum allowed to improve the aerobic stability both in laboratory silos and bunker silos, the magnitude of results obtained was different. Yeast count was lower, and aerobic stability was higher in bunker than in laboratory silos, despite lower acetic acid concentrations in bunker than laboratory silages were detected. It was hypothesized that some unrevealed factors, other than acetic acid content, can contribute to the aerobic stability of bunker silages.

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The Use of Bale Compactor to Produce High Density Wrapped Bales of Several Forages and Grains

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Keywords: high density bale compactor, high moisture corn, Italian ryegrass, wheat, sorghum

Introduction

The need to ensile fine chopped forages, milled grains, crop residues and by-products has become important to reduce the production costs of milk and meat, especially for small to medium farms, where bunker management poses high risks for aerobic deterioration. The baler-wrapper combined machines introduced in the European market in 2013 that are able to bale many different materials, such as corn silage, high moisture grain silage, grass and legume chopped forages, sugar pulp, total mixed rations (TMR), etc. The formed bales are tied with plastic film and then wrapped with stretch film. Many of these new machines have adopted film tying in the compression chamber and two film dispensers to speed up the wrapping operation, and showed to produce bales with dry matter (DM) densities above 300 kg/m³, with peaks of 600 kg/m³ for high moisture corn silages. The aim of this study was to assess the fermentation characteristics, microbial profiles, aerobic stability, and DM density of forages and grains baled on farm with compactors under different DM contents and lengths of conservation periods.

Materials and methods

The survey was carried out over a two-year period on farms that have adopted ensiling in wrapped bales produced with agricultural compactors (such as Dens-X Orkel or LT-Master Goweil) secured with polyethylene (PE) film and wrapped with 8 to 9 layers of PE stretch film. Different products were considered: whole crop cereals, i.e. corn (*Zea mays*), sorghum (*Sorghum bicolor* and *Sorghum sudanense*), winter cereals (*Triticum aestivum*, *Triticum secale*); wilted chopped forages, i.e. alfalfa (*Medicago sativa*), Italian ryegrass (*Lolium multiflorum*), and permanent meadows; high moisture ear corn (*Zea mays*), and total mixed ration (TMR). A total of 140 bales of the different products, each representative of homogeneous lots of 30 to 70 bales, were sampled and analyzed. The bales were weighted, at baling and at immediately before opening, to determine the fresh and DM densities and weight losses. The silages were analyzed for DM content, pH, fermentative profile by HPLC, microbial count of yeast and mold, and aerobic stability. Aerobic stability was determined under controlled conditions and defined as the number of hours the silage remained stable before rising more than 2°C above ambient temperature.

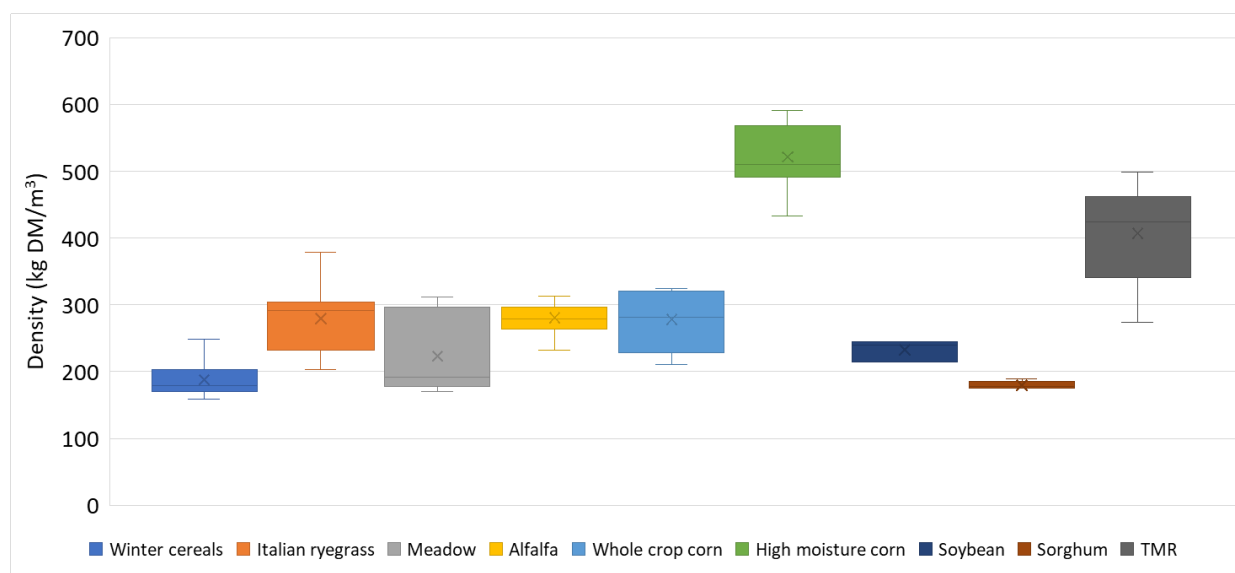
Results

The silages produced presented a wide range of DM content (from 19 to 78% for forages, and from 53 to 69% for whole ear corn silages), pH, and conservation period (from 15 to 433 d). DM densities ranged from 159 to 249 kg/m³ for direct cut winter cereals, from 170 to 379 kg/m³ for wilted forages, from 175 to 325 kg/m³ for whole plant corn and whole plant sorghum, from 433 to 591 kg/m³ for whole ear corn silage, and from 274 to 499 kg/m³ for TMR, respectively (Figure 1). The yeast count was found to be higher than 3 log cfu/g in less than 33% samples (47 out of 140). The aerobic stability was highly variable in relation to ensiled material, DM content, and length of conservation, and ranged from few hours (12 samples out of 140) to higher values than 500 h. However, most of the samples conserved for more than 30 d, were characterized by aerobic stability higher than 100 h. When the DM content was adequate for the conservation of the single category of ensiled material, the fermentation profiles showed a dominant lactic acid fermentation with pH comparable or even lower than those commonly observed in silages conserved in horizontal silos at similar conditions. Butyric acid fermentation profiles were rarely observed, especially in very low DM content silages such as direct cut wheat and Italian ryegrass silages wilted less than 24h. Weight losses were always very low, ranging from 0.68% to 5.06% of the original bale weight. The costs for harvesting, baling, and wrapping ranged from 16 to 20 € per bale, that means a cost for a ton of DM ranging from 26 to 92 €, in relation to the amount of DM stored in each bale.

Table 1. Main characteristics of the silages analyzed (n =140). Minimum and maximum value in brackets.

Silage	DM (%)	pH	Lactic acid (g/kg DM)	Acetic acid (g/kg DM)	Butyric acid (g/kg DM)	Ethanol (g/kg DM)	1,2 propandiol (g/kg DM)	Yeast (log cfu/g)	Aerobic stability (h)
Winter cereals	29 (25-32)	3.83 (3.61-4.27)	55.4 (12.9-79.0)	27.5 (11.0-58.3)	7.2 (<0.01-48.5)	31.3 (22.2-45.3)	10.2 (0.2-21.3)	2.64 (0.50-8.70)	127 (30-260)
Italian ryegrass	43 (19-68)	4.48 (4.12-4.94)	53.4 (24.9-75.8)	13.2 (2.9-26.3)	4.2 (<0.01-71.4)	3.6 (<0.01-14.3)	1.6 (<0.01-7.2)	1.8 (0.50-3.79)	527 (115-1028)
Alfalfa	53 (31-78)	4.97 (4.37-5.85)	33.7 (<0.01-95.6)	7.8 (<0.01-31.4)	<0.01	3.2 (<0.01-10.6)	0.1 (<0.01-0.7)	1.86 (0.50-4.00)	309 (309-309)
Permanent meadow	29 (22-44)	4.13 (4.00-4.28)	38.5 (14.7-48.8)	12.1 (3.0-18.2)	<0.01	5.9 (2.1-9.2)	1.5 (<0.01-3.6)	1.99 (0.50-4.23)	-
High moisture corn	58 (53-69)	4.02 (3.72-6.21)	24.6 (<0.01-37.9)	4.4 (<0.01-8.8)	0.02 (<0.01-1.5)	5.9 (<0.01-13.5)	0.4 (<0.01-6.4)	4.1 (0.50-8.08)	109 (26-345)
Corn silage	38 (35-42)	3.7 (3.52-3.78)	41.9 (<0.01-58.9)	7.6 (<0.01-12.2)	<0.01	12.3 (<0.01-19.5)	0.6 (<0.01-2.9)	2.89 (0.50-7.32)	120 (26-345)
Sorghum	46 (29-58)	4.84 (4.56-4.97)	9.03 (7.1-11.3)	4.8 (4.2-6.7)	<0.01	23.2 (19.9-29.2)	<0.01	-	-
TMR	52 (48-55)	4.85 (4.31-5.24)	32.6 (16.8-57.4)	21.1 (11.5-32.8)	<0.01	6.03 (2.85-19.3)	<0.01	4.11 (0.50-6.83)	-

cfu = colony forming units; TMR = total mixed ration for dairy cow.

**Figure 1.** Boxplot of DM densities of different silages ensiled in high-density bales.

Conclusions

Agricultural compactors offer a wide flexibility for small to medium farms that have too low daily silage consumption to be conserved in horizontal silos. The bales showed high forage density, fermentation quality, and aerobic stability even higher than those observed on farm bunker silos in similar DM content and length of conservation period. This highly efficient method of conserving forages permits to valorize different home-grown crops without having problems of contemporary managing several feed-out silo faces and/or silos of inappropriate dimensions.

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Microbial Dynamics and Cooperative Fermentation: Insights from Real-Time Monitoring of Silage Processes

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Keywords: Real-Time pH Monitoring, Silage Microbiome Dynamics, LAB Cooperation

Introduction

The microbiome of silage before, during, and after ensiling has been well studied. Most studies investigate the microbial community at pre-defined time intervals (f.e. Keshri et al., 2018), which, however, does not directly represent the phases of ensiling. This can be overcome by using bioreactors that monitor the pH development during silage fermentation in real-time (Shan et al., 2021), which allows real-time assessment to sample the reactor for microbiome analysis. Based on this, the microbiota responsible for the main fermentation phase can be revealed. Considering this information, candidates for effective inoculants can be preselected. A rapid pH-drop due to lactic acid production by lactic acid bacteria (LAB) inhibits undesired microorganisms, and an acetic acid built-up by heterofermentative LAB increases aerobic stability by suppressing yeast growth (Bolsen et al., 1996; Danner et al., 2003). However, heterofermentative degradation of hexoses also yields in ethanol production, reducing silage quality (Kung et al., 2018) but this can be shifted to acetic acid production if additional electron acceptors are present for NAD(P)H reoxidation (Zaunmüller et al., 2006). Growth in a co-culture instead of pure culture might supply these redox partners through metabolites by other microorganisms. Therefore, this study aims to isolate representative strains of populations defined as relevant based on comprehensive microbiological methods, including full gene sequence 16S amplicon analyses to samples taken according to the real-time pH-developments of three types of silage (grass, alfalfa and maize). Subsequently, cooperation between these isolates was investigated to suggest potential inoculants, which produce lactic and acetic acid while minimizing ethanol production.

Materials and methods

Grass, alfalfa, and maize were ensiled in twelve 1.5 L jars, nine of which were bioreactors with real-time pH monitoring (Sun et al. 2024). Silage sampling occurred at five points in time, covering the fresh material (t₀), the main fermentation phase (t₁–t₃), and the silage in stable storage (t₄). The samples for t₁-t₃ were taken from the vessels equipped with pH sensors. At each sampling point in time, three of the reactors were opened and processed. The analysis included microbial counts (total, enterobacteria, yeasts, and LAB) and the microbial composition of complex silage samples, using both an improved cultivation-dependent method and cultivation-independent long-read amplicon sequencing of the 16S rRNA gene (using Pacific Biosciences (PacBio) single-molecule real-time sequencing (SMRT) platform). An optimized cultivation medium was created for the cultivation-dependent method, and about one hundred isolates from each sample were identified. Isolates from silage samples were selected according to PacBio sequence data for growth experiments performed in pure and co-cultures. Metabolites were determined using HPLC to investigate whether co-cultivation yields in higher acetic acid and lower ethanol amounts by cooperation effects between co-cultivated strains.

Results

Based on the pH curves of each silage and the data obtained by cultivational methods and PacBio SMRT technology, it could be observed that in all silages the main fermentation phase is dominated by heterofermentative bacteria belonging to the genera *Leuconostoc* and *Weissella* as well the homofermentative species *Lactococcus lactis*. In the later phase t₄, after stabilization of the pH-value, the predominant bacteria belong to the genera *Lactobacillus*, *Lentilactobacillus*, *Lactiplantibacillus*, *Levilactobacillus* and *Pediococcus* (figure 1), comprising heterofermentative and homofermentative LABs. The investigation of cooperations between strains isolated from these samples revealed that co-cultivation of specific combinations of heterofermentative LAB and homofermentative representatives yields a lower production of ethanol and a greater production of either acetic or lactic acid.

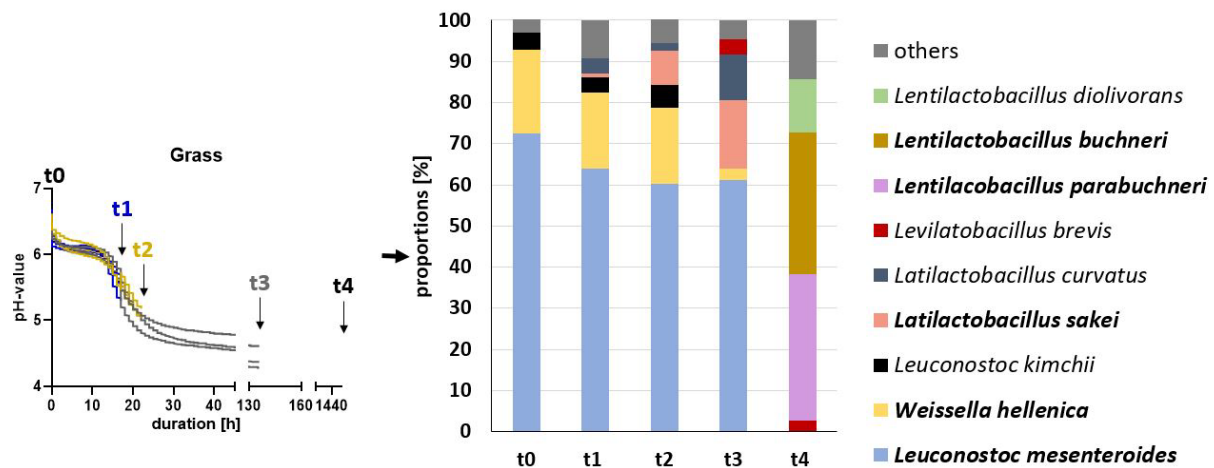


Figure 3: real-time pH curve of grass silage and associated microbial community based on cultivational investigations. Sampling occurred 0 hours (t0), 17 hours (t1), 22 hours (t2), 1 week (t3) and two months (t4) after ensiling, with pH-monitoring between t0 and t3. Bold species names indicate main representatives.

Conclusions

The results indicate that fermentation of silage is mainly driven by heterofermentatives such as *Leuconostoc mesenteroides* and *Weissella hellenica*. The cooperation of heterofermentative with homofermentative LAB yields lower ethanol concentrations and higher amounts of organic acids. Although the details of the cooperative mechanisms, especially the increased lactic acid production, still need to be elucidated, the identification of performance-enhancing strain combinations is important for the future creation of new inoculants.

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Approach to Adjust Acid Application Using pH Measurements Prior to Ensiling

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Keywords: additive optimization, buffering capacity, organic acids, acid-base titration.

Introduction

In Northern Europe, formic acid-based silage additives are widely employed to ensile challenging forages with low dry matter (DM) and water-soluble carbohydrate contents, including swards dominated by forage legumes and timothy (*Phleum pratense*). These additives effectively lower silage pH, suppress excessive fermentation, and inhibit the growth of undesirable microorganisms. When combined with substances such as propionic acid, sorbates, or benzoate, formic acid-based additives also enhance silage aerobic stability. For dairy farmers, the use of such additives offers multiple benefits, including increased milk yield, improved milk fat and protein content, and minimized losses during the ensiling and feed-out phase (Wilkinson and Rinne 2018).

Application guidelines for acid-based additives are typically provided in liters per ton of fresh forage. However, many farmers lack access to drive-over scales, which often results in inaccurate dosing, either under- or overdosing. Underdosing, particularly in low DM silage, can lead to undesirable outcomes such as excessive lactic acid fermentation or high levels of volatile fatty acids. These factors negatively impact feed intake, animal performance, and in extreme cases, animal health. Furthermore, milk produced from animals fed poorly preserved silage may be rejected by dairy companies due to off-flavors or elevated spore counts. Therefore, developing approaches that help farmers enhance the precision of additive application is of significant importance.

Handheld pH meters, which are now affordable and user-friendly, offer a promising solution. Measuring the pH of acid-treated forage before ensiling could provide a practical and biologically relevant tool to adjust additive application rates. However, for widespread adoption of this approach, several prerequisites must be met. Farmers require clear instructions for accurate pH measurements under farm conditions, as well as pH target values for different forage types, whether fresh or prewilted.

Forage buffering capacity, which reflects its resistance to pH change, can be measured by titration with lactic acid (Weissbach et al. 1974). This approach can be utilized, but may require adaptation to ensure that sample storage and processing effects do not skew results compared to pH measurements taken in farm conditions. The present study investigated the effects of sample storage and preprocessing on titration results of timothy and red clover (*Trifolium pratense*) using lactic and formic acids. The study aims to provide foundational knowledge for developing new accurate and practical recommendations for formic acid-based silage additives.

Materials and methods

This experiment was conducted at the Natural Resources Institute Finland (Luke) in Jokioinen, Finland (60°48'N, 23°29'E). Samples of timothy at early heading stage and blooming red clover samples, either fresh or frozen, were subjected to two levels of mechanical processing: manual chopping using scissors or crushing using a blender. To extract soluble components, 20 g of processed sample was mixed with 300 mL of distilled water. The mixture was subsequently filtered to remove particulate matter, and 40 mL of the filtrate was transferred to the titration unit of titrator (Titrator Excellence T5, Mettler Toledo, Hamilton, New Zealand). Two titrants, 1N solutions of formic and lactic acids, were used. Acid was gradually added to the filtrate while pH changes were recorded. In the analysis of variance, dependent variable was the acid volume required to reach pH of 4, while the independent variable was the interaction between acid type, crop type, storage condition, and processing method. Number of replicates was three. Pairwise comparisons were conducted using Tukey's test at a significance level of 5%.

Results

The differences between formic acid and lactic acid during titration reflected their distinct dissociation constants (pK_a). Formic acid, having a lower pK_a value, is a stronger acid than lactic acid. The relative difference detected in the consumption of 1N titrant is doubling when expressed on an acid mass basis. Although red clover is generally considered to exhibit higher buffering capacity than grass species, this distinction was evident only when fresh samples were titrated with lactic acid. Additionally, the difference between manual processing and using a blender was apparent only in fresh samples. However, freezing the samples prior to titration eliminated differences between crops and processing methods, likely due to disruption of cell wall structures caused by freezing and melting.

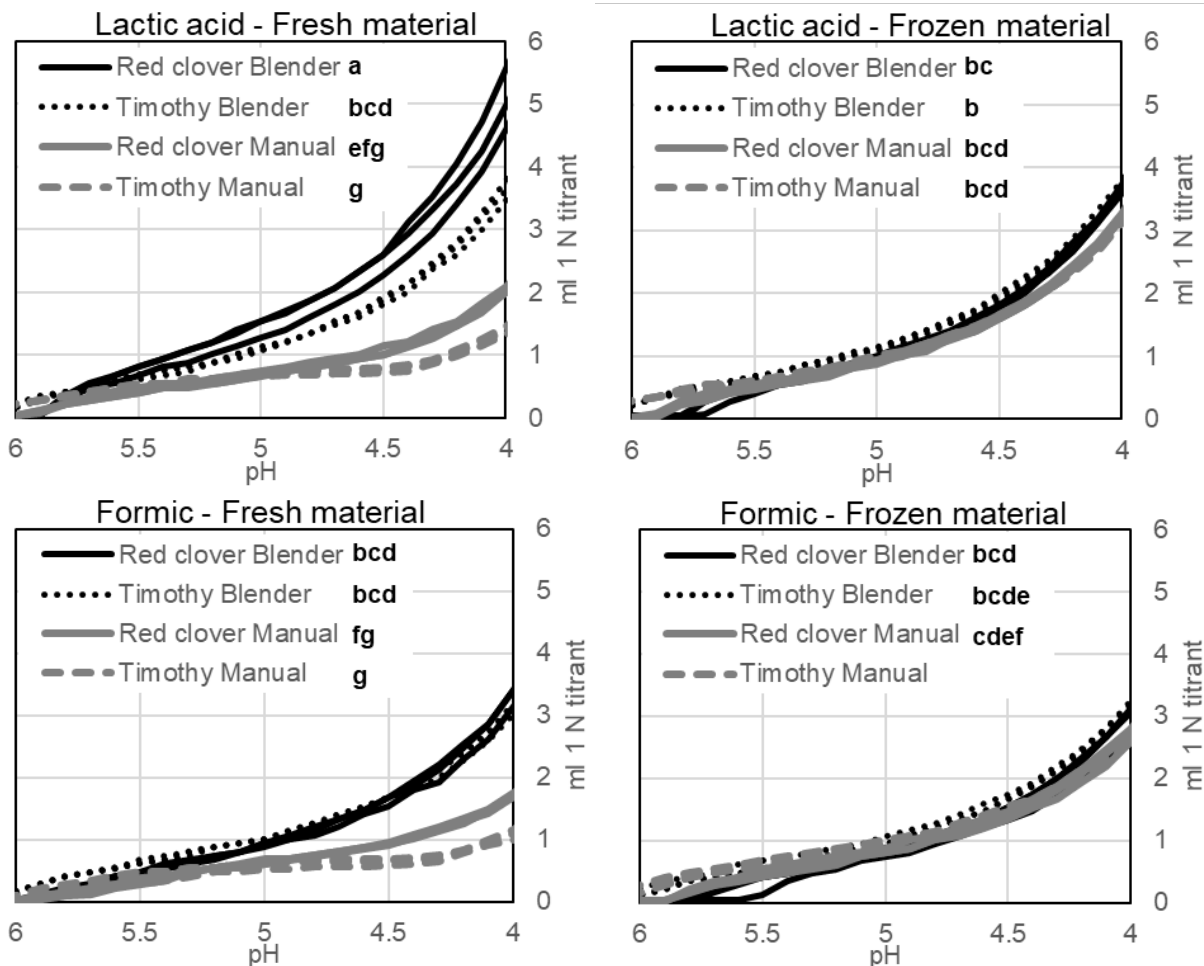


Figure 1. Consumption of 1N acids during titration of filtrates from red clover and timothy samples. Tukey's test letters indicate significant ($p < 0,05$) differences among treatment means.

Conclusions

Recommendations are needed to advise farmers to which extent pH should decrease when using formic acid-based additives, assuming that pH measurements are taken immediately after grass loads arrive at the silo. These recommendations should be created considering the preprocessing level of grass that can be performed near the silage site within a relatively short time. Since cell wall eventually breaks down during ensiling process, it is worth considering how this factor will ultimately be taken into account when developing practical guidelines.

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Control of Regulated and Emerging Silage Mycotoxins

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Keywords: mycotoxin contamination, prevention strategies, management practices

Introduction

Mycotoxins are defined as a group of secondary metabolites produced by several fungal species belonging to the genera *Fusarium*, *Aspergillus*, and *Penicillium* spp. and a lot of other mycotoxigenic fungi (Gallo et al., 2024). These compounds have the peculiarity to be chemically stable to physical or chemical treatments and they can be detected in a plethora of raw materials, by-products as well as homegrown forage, included silage (Gallo et al., 2015; Ogunade et al., 2018). In particular, the contamination of silage can be related to several factors that are referred to forage species, stage of maturity of crop at harvest, ensiling process, storage site, use of silage additives, feed-out and bunk management conditions (Mahanna and Chase, 2003). Recently, researchers have isolated bacteria, yeast, and fungi that are able to detoxify mycotoxins. For instance, lactic acid bacteria (LAB) perform an effective role in mycotoxins deactivation in silages due to their important role during the ensiling process (Li et al., 2021). This contribution aims to present the risk of silage contamination by regulated and emerging mycotoxins that can concomitantly contaminate silage. Further, it would describe the main strategies applied to prevent mycotoxin contamination on farm, in order to reduce economic loss and define strategies to minimize the risk of mycotoxin contamination in lactating dairy cows.

Strategies to prevent mycotoxin contamination in ensiled crop

Proper ensiling practices (e.g., the DM concentration of the crop, define theoretical length of cut, filling and packing operations, rapid sealing operation) allow to reduce or prevent the occurrence of mycotoxins and the growth of undesirable microbes, both in field and after harvest conditions. About use of bacterial inoculants, several studies have investigated the effects of these additives on improving silage fermentation and aerobic stability or reducing fungal growth and mycotoxin productions (Dalié et al., 2010; Ranjit and Kung, 2000; Oliveira et al., 2017). Despite the topic is still debated, many authors (Oluwafemi et al., 2010; Ma et al., 2017; Gallo et al., 2018, 2021) observed that LAB can prevent mycotoxin contamination of silage by detoxifying or deactivating mycotoxin mother molecules *in vitro*. Particularly, the LAB species identified as potential agents to prevent or inhibit the growth of toxic fungi were *Lactococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc* genera.

Interesting results related not specifically to mycotoxins, but to silage safety aspects, was from Mosconi et al. (2023), who investigated the effect of management practices related to silage and total mixed ration (TMR) residues on a reservoir of *Clostridium tyrobutyricum* contamination in the preparation of TMR. Improper management practices of both residues can lead to higher contamination of such kind of butyric acid bacteria. Therefore, proper management practices, such as disposal of the spoiled silage from the upper layer of the bunker and a suitable cleaning of the mixing wagon, may allow to limit *Clostridium tyrobutyricum* contamination in these samples as well as TMR. Rapid monitoring contamination system applied to animal feed may help to prevent economic losses and maintain animal health. Notably, Ghilardelli et al. (2022) through the near-infra-red spectroscopy (NIRS) performed a calibration using a Random Forest model. The results indicated satisfactory classification of samples after considering different cut-offs to classify samples referring to mycotoxin concentration ($\mu\text{g/kg DM}$) or count (n): i) total detectable mycotoxins; ii) regulated and emerging *Fusarium* toxins; iii) emerging *Fusarium* toxins; iv) fumonisins and their metabolites; and v) *Penicillium* toxins. This technique can not directly determine the levels of different mycotoxins and fungi, even if it can provide important information regarding risk of regulated and emerging contamination levels into corn silage.

Mycotoxin exposure and strategies to minimize the risk of contamination

The effects of regulated and emerging mycotoxins after their ingestion may vary, depending on the chemical structure and the amount of mycotoxins, considering the time of exposure and animal susceptibility (e.g., species, age, immune status, gender) (Yu and Pedroso, 2023; Okechukwu et al., 2024). Several trials investigated the effects of co-exposure to mycotoxins, mainly *Fusarium*-produced toxins (e.g., DON and ZEN), which may be often detect in ruminant diets (Gallo et al., 2022). Catellani et al. (2023) investigated the effects of an exposure period of 54 days to *Fusarium* mycotoxins on dairy cows' performance and health. No differences in terms of DM intake were observed between control and mycotoxin contaminated diets. On the other hand, lower milk production was reported for cows fed the treated diet in comparison to the control (i.e., 37.6 vs 38.2 kg/day, respectively). Decreased intake and milk production, diarrhea, and reproductive failure were observed in primiparous or multiparous lactating dairy cows fed diets contaminated with 366 $\mu\text{g/kg DM}$ of ZEN and 1141 $\mu\text{g/kg DM}$ of DON (Catellani et al., unpublished).

In the last year, trials (Gallo et al., 2020; Catellani et al., 2023) have suggested that the inclusion of adsorbent materials in animal diets is a reliable method for preventing mycotoxins contamination in farms. One option is represented by mycotoxin sequestering agents (i.e., compounds able to bind mycotoxins in contaminated feeds). Overall, the toxin-sequestering agent complex passes through the gastrointestinal tract of the animal, and the toxin can finally be eliminated via excretion (feces) (Masoero et al., 2009; Ahn et al., 2022). The application of adsorption binders, such as silica-based inorganic compounds or carbon-based organic polymers, have been investigated for their capacity to adsorb mycotoxins (Liu et al., 2022). Clays are applied as inorganic binders for reducing AFB1 intoxication of livestock, and the AFM1 carry over in milk (Gallo et al., 2015). Notably, chemically inert bentonite clays

have been suggested to be the best adsorbents for aflatoxin sequestration (Hamad et al., 2023). On the other hand, there is a lack of information about the absorbing efficiency of these binders against *Alternaria*, *Aspergillus*, *Penicillium*, and *Monascus*-derived toxins.

Conclusions

To prevent the adverse effects of mycotoxin ingestion in cattle, farmers have to adopt the best practices to grow and harvest crops in the field, to ensile forages with the purpose to reduce the zone exposed to the risk of air penetration and aerobic instability. Future researches are needed to investigate sequestering efficiency or bioactivating ability of different products against many *Alternaria*, *Aspergillus*, *Penicillium*, and *Monascus*-derived toxins that are usually detected in forages, including use of bacterial inoculants.

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Comparison of Techniques to Assess Grain Processing in Whole-plant Corn

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Keywords: corn silage, grain processing, kernel processing score, sieving

Introduction

The harvesting process significantly influences the quantity and availability of nutrients in whole-plant corn silage (WPCS), being affected by variations in the types and settings of the equipment used (Ferraretto et al., 2018). Corn silage processing score (CSPS) has been used as a proxy of kernel breakage in corn silage, as it measures the proportion of starch passing through a 4.75 mm sieve after vertical agitation separation procedures using dry WPCS samples (Ferreira and Mertens, 2005). However, CSPS does not allow for decision-making at harvest. Therefore, the objective of this study was to compare lab and on-farm techniques to measure kernel processing in whole-plant corn forage.

Materials and methods

Sixty samples of fresh chopped whole-plant corn of various hybrids, maturity stages, cutting heights, forage harvesters, chopping lengths, and kernel processors were collected from commercial farms in the South and Southeast regions of Brazil between 2023 and 2024. Samples were analyzed for chemical composition, CSPS (% starch passing through a 4.75 mm sieve), and number of intact kernels (n per 250 g as fed, considering >3/4 pieces and whole grains). Additionally, from each fresh sample, three subsamples were used for hydrodynamic separation of the grain fraction. Hydrodynamic separation involves mixing fresh chopped whole-plant corn in water to allow the less buoyant kernel particles to sink, while the more buoyant plant particles float. This method is effective at separating grain from stover in fresh material but drying the samples before water separation yields a more complete separation. One grain fraction was spread on a black surface to capture an image by the Silage Snap app. Grain fraction from the second subsample was dried using a kitchen convection oven (air fryer) followed by manual sieving using a 4.75-mm sieve. A third grain sample was dried for 48 h in an oven at 55 °C followed by sieving using a Ro-Tap shaker (including sieves with nominal square apertures of 8.00, 6.70, 4.75, 3.35, 2.36, 1.70, 1.18, and 0.60 mm and pan) to calculate the geometric mean particle size (GMPS) and the proportion of kernels passing through a 4.75-mm sieve. The strength of a linear relationship between two techniques was assessed by Pearson correlation using the CORR procedure of SAS. Regressions were established between the proportion of kernels passing through a 4.75-mm sieve measured by on-farm (manual sieving and Silage Snap app) and on-lab (Ro-Tap shaker) techniques using the REG procedure of SAS. Lin's concordance correlation coefficient (CCC) was calculated for each regression model.

Results

Overall, whole-plant corn forage samples contained (mean $\pm$ SD) 37.6 $\pm$ 5.22% DM, 43.3 $\pm$ 7.14% aNDFom, and 28.1 $\pm$ 7.78% starch. Regression analyses showed that the proportion of kernels passing through a 4.75-mm sieve measured by on-farm techniques were positively correlated with the on-lab measurement, but the CCC revealed a stronger concordance between the Ro-Tap shaker (on-lab) with manual 4.75-mm sieve than that with Silage Snap app (Figure 1). Furthermore, using a manual 4.75-mm sieve is easier than sorting each individual grain particle for taking a picture. Considering that GMPS provides a better assessment of kernel fragmentation (Dias Junior et al., 2016) and that CSPS is widely used to assess (indirectly) grain processing in WPCS, those values were compared with all other kernel breakage indexes by Pearson correlation (Table 1). Correlation coefficients indicated that the proportion of kernels passing through a 4.75-mm sieve at the Ro-Tap shaker related well with the GMPS ($r = -0.944$). Among the on-farm indexes, manual sieving using a 4.75-mm sieve was fairly correlated with GMPS ($r = -0.831$). The number of intact kernels ($r = -0.700$) and the proportion of kernels < 4.75-mm estimated by Silage Snap app ($r = -0.688$) were also reasonably correlated with GMPS. However, the agreement between intact kernels and GMPS decreased with low numbers of intact kernels (not shown). Parallel relationships between kernel breakage indexes and CSPS were always poorer than with GMPS suggesting that the direct measures were better than the CSPS proxy.

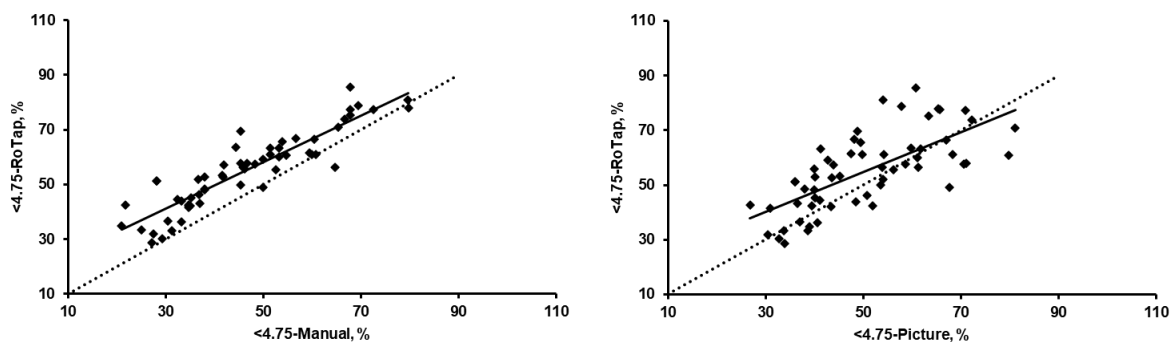


Figure 1. Relationships between the proportion of kernels passing through a 4.75-mm sieve measured on lab by Ro-Tap shaker (<4.75-RoTap) and on farm by manual sieving (<4.75-Manual) or Silage Snap app (<4.75-Picture) techniques. $<4.75\text{-RoTap, \%} = 15.5 + 0.853 \times (<4.75\text{-Manual})$; $P < 0.001$; $\text{RMSE} = 5.91$; $R^2 = 0.823$; $\text{CCC} = 0.764$. $<4.75\text{-RoTap, \%} = 18.3 + 0.727 \times (<4.75\text{-Picture})$; $P < 0.001$; $\text{RMSE} = 10.1$; $R^2 = 0.484$; $\text{CCC} = 0.659$. Dotted line indicates $Y = X$.

Table 1. Correlations[†] among kernel processing indexes in fresh chopped whole-plant corn (n = 60)

Item	CSPS, %	<4.75-RoTap [‡] , %	<4.75-Manual [‡] , %	<4.75-Picture [‡] , %	Intact kernels ⁴ , n 250 g ⁻¹
GMPS, μm	-0.637	-0.944	-0.831	-0.668	0.700
CSPS, %		0.694	0.707	0.470	-0.368

[†] $P < 0.01$ for all correlation coefficients.

[‡]Proportion of kernels passing through a 4.75 mm sieve measured using a Ro-Tap shake on lab (<4.75-RoTap), manual sieving on farm (<4.75-Manual), or Silage Snap app on farm (<4.75-Picture).

Conclusions

On-farm manual sieving of the grain fraction with a 4.75-mm sieve, combined with hydrodynamic separation, is an effective tool for evaluating kernel breakage, aiding in decision-making during harvesting of WPCS.

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Effect of Environmental Factors on Aerobic Stability of Whole-plant Corn Silage Inoculated with *Acetobacter pasteurianus*

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Keywords: *Acetobacter pasteurianus*, whole-plant corn silage, aerobic stability, bacterial community, response surface methodology.

Introduction

Silage in hot and humid regions often suffers from unstable fermentation, dominated by acetic acid production due to heterofermentative LAB, *enterobacteria*, and *Acetobacter* bacteria. *Acetobacter* is commonly regarded as an indicator of aerobic deterioration in silage [1], yet its precise role in fermentation and during aerobic exposure, as well as the factors that promote its growth, remain insufficiently understood. The study investigated the impact of environmental factors on silage quality and the proliferation of *A. pasteurianus*, using response surface methodology (RSM) to identify strategies for improving aerobic stability in such conditions.

Materials and methods

Whole-plant corn (*Zea mays* L.) plants of the variety AoYu 005 were harvested when it reaches the 1/2 milk-line stage, and processed into 1-2 cm pieces with dry matter (DM) contents adjusted to 30%, 25% and 20%. Silage materials were inoculated with *A. pasteurianus* (10^6 cfu·ml⁻¹, CGMCC No. 1.1810) at concentrations of 40%, 50%, and 60%, vacuum-sealed, and stored at 20°C, 30°C, and 40°C for 60 days. Following the anaerobic storage phase, the silage was exposed to air at room temperature (20-25°C) for 7 days, both with and without *A. pasteurianus* inoculation. Each treatment had three replicates. The quality, aerobic stability, and microbial community of silage were tested. The qPCR was conducted to confirm treatment difference in relative abundance of *A. pasteurianus* between treatments.

Results

The results demonstrated that *A. pasteurianus* did not impact the nutritional value of the silage during anaerobic fermentation, maintaining a low pH (< 3.80). However, the presence of *A. pasteurianus* significantly reduced the aerobic stability of the silage. The microbial community shifted from primarily *Klebsiella* species initially to *Lactobacillus* and *Acetobacter* species post-ensiling. During the aerobic exposure phase, *A. pasteurianus* and *A. fabarum* became the dominant species. RSM analysis demonstrated that at a temperature of 28°C, a DM content of 25%, and an initial *A. pasteurianus* concentration of 5.2×10^6 cfu·ml⁻¹, the highest concentration of *A. pasteurianus* was observed during the aerobic exposure stage.

Conclusions

The presence of *A. pasteurianus* and *A. fabarum* in whole-corn silage was a natural occurrence in hot and humid areas. During the conventional fermentation stage, *A. pasteurianus* did not negatively impact the quality of whole-corn silage when properly sealed. However, a transition from anaerobic to aerobic conditions resulted in a rapid decline in the aerobic stability of the silage inoculated with *A. pasteurianus*. The presence of *A. fabarum* did not appear to exert a detrimental impact on the aerobic stability of the silage. Future efforts should be focused on the prevention and control of *A. pasteurianus* in whole-plant corn silage to prolong aerobic stability in hot and humid regions.

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Nutrient Distribution of White Corn Silage and Dispersion Analysis of Samples Analyzed with NIR

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Keywords: Nutrient distribution, white corn silage, dispersion analyzed

Introduction

White corn silage (WCS) is the main forage used in the feeding of dairy cattle in central Mexico. Although its energy contribution is essential for the creation of diets for dairy cows (1,2,3), there is very little information on WCS (4). It is reported by the Secretaría de Agricultura y Desarrollo Rural that in 2020, 7 million Hectares of white corn (92.7%) were planted against 553 thousand Hectares of yellow Corn (7.3%) in Mexico (7).

Because the WCS has more participation in dairy cattle diets in Mexico, it is important to know the distribution of each nutrient and the possible correlations that may exist among nutrients and forage dry matter to understand the relationships between variables (3,4,5). The statistics of 8,679 samples from the past 5 years of WCS offer unique information relative to the Central area of Mexico. The purpose of this study is to gain a more accurate understanding of the main statistics and correlations between the main nutrients of WCS in central Mexico, serving as a technical tool in the formulation of diets for ruminants.

Materials and methods

The data used for this study are the results of NIR analysis of Corn Silage by Laboratorio Servicios GAQ S.A de C.V., an affiliate of CVAS (Cumberland Valley Analytical Services). The Laboratorio Servicios GAQ S.A. de C.V. is certified by the NFTA (National Forage Testing Association).

The information is from 2020 to 2024. The statistical analyses focus on dry matter (DM), crude protein (CP), acid detergent Fiber (ADF), neutral detergent Fiber (NDF), digestibility of the NDF at 30 hr. (6). There were 8,679 corn silage samples analyzed, with the results almost completely from WCS. Statistical analyses were performed using the Minitab Workspace software version 21.1.0. Both S and R² determination are used nutrient dispersions.

S Formula

R² Formula

$$S = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{n - 2}}$$

$$R^2 = 1 - \frac{SSE}{SST}$$

Results

Table 1. Values for main analytes of corn silage from central Valley of Mexico 2020-2024, dry matter basis.

	Dry Matter	Crude Protein	ADF	NDF	Digestibility of NDF 30 hrs. % NDF	Starch
Years 2020 - 2024						
Average	32.96	7.93	29.47	46.53	57.85	22.54
Estandar Deviation	5.69	1.11	3.34	5.05	4.18	6.24
Max – Min Values	59.80-16.90	14.2-4.2	50.4-20.0	76.0-26.4	77.9-21.66	43.5-3.0
Number of samples	8,674	8,697	8,696	8,699	8,685	8,620

Table 2. Values of dispersions between the nutrients of Corn Silage; Crude Protein, ADF, NDF, Dig. NDF 30 h % NDF and Starch. Statistical result of the value of S and R² in the dispersion, where the column is the variable y and the rows the variable x, the correlations are made between all the main nutritional values.

	CP		ADF		NDF		Dig. NDF 30 h % NDF		Starch	
	S	R-square	S	R-square	S	R-square	S	R-square	S	R-square
Dry Matter	1.062	8.74%	2.859	26.80%	4.344	25.76%	5.636	1.83%	4.768	28.70%
Crude Protein	-----	-----	1.071	6.99%	4.741	11.82%	1.019	15.69%	0.979	17.67%
ADF	-----	-----	-----	-----	1.125	88.67%	3.337	0.31%	1.591	74.85%
NDF	-----	-----	-----	-----	-----	-----	4.99	2.01%	2.184	79.25%
Dig. NDF 30 h % NDF	-----	-----	-----	-----	-----	-----	-----	-----	3.931	8.33%
Starch	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

S = It is a measure that indicates how far away, on average, the actual values of the data are from the values predicted by the regression calculate. The lower the S-value, the better the calculation fits the data, as the points are closer to the regression line.
R² or square = It is a metric that measures what proportion of the total variability of the dependent variable (response variable) is explained by the calculate.

Conclusions

The analysis done in this study shows a confidence interval of 95% on the mean, median and standard deviation on the data for nutrient distribution. This demonstrates that the samples analyzed (8,699) using statistical tools do not have a significant dispersion. You might expect about 95% of the samples to produce ranges containing the

population parameter. The precision of this data will offer a greater certainty on the formulation of diets for dairy cows in Central Mexico.

The results of R^2 and S are accurate because of having large samples in the corn silage analysis (Minitab Support version 21.1.0), high values of R^2 and a low number of S ($>$ to 60 % for R^2 and $<$ to 3 in S, 60 – 80 % R^2 of strong relationship) in the dispersions made with all the nutrients of Corn Silage were; NDF – ADF 88.67 % R^2 , 1.12 S, Starch – NDF 79.25 % R^2 , 2.18 S and Starch - ADF 74.85 % R^2 , 1.59 S. In general, the higher the value of R^2 , the greater the strength of the relationship between the 2 variables, 60 – 80 % R^2 is strong relationship.

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Nutritional Value and Ruminal Digestibility of Different Sorghum Forage Types at Two Growth Stages

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Keywords: Brown-midrib, sorghum, sudangrass, millet

Introduction

Sorghum (*Sorghum bicolor*), sudangrass (*Sorghum bicolor* ssp. *drummondii*), and their hybrids are viable alternatives to corn silage, providing more flexibility for warm-season forage production. Unlike corn silage, grain contribution in sorghum silage is minimal. Delaying harvest increases sorghum forage biomass, but this comes at the cost of digestibility due to higher lignin content. Therefore, selecting low-lignin sorghum varieties can help achieve high biomass production with better nutrient value silage. Naturally occurring brown midrib (*bmr*) mutations improve the energy value of warm-season annual forage crops by reducing lignin synthesis. Harvesting *bmr* sorghum at a more advanced growth stage can result in more digestible forage. A study compared *bmr* and non-*bmr* forage sorghums to evaluate potential gains in nutrient value and digestibility at the pre-boot and half-bloom stages.

Materials and methods

Brown midrib types warm-season annual forage crops (three forage sorghum, two sorghum-sudangrass hybrids, and two sudangrass) were sampled along with non-*bmr* type warm-season annual forage at the pre-boot stage and half-bloom stage. A grab sample of approximately 300 g from each type was taken to measure crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and in vitro true digestibility (IVTD). The collected biomass samples were dried at 55° C for 72 h and milled to pass a 2-mm screen using a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA), then ground to pass a 1-mm screen using a cyclone mill (Udy Corporation, Fort Collins, CO, USA). The CP was measured by a combustion method (Bremner, 1965). The ADF and NDF were determined according to Goering and Van Soest (1970), and lignin content was determined as acid-detergent insoluble lignin (AOAC 1990). Ruminal digestibility and digestion parameters were measured (Van Soest, 1970; Weimer et al., 2003). Measured nutritional indexes were analyzed using the Proc Glimmix of SAS version 9.4 (SAS Institute, 2016), considering sorghum type, growth stage, and interaction between type and growth stage as fixed effects. The significance of fixed effects was determined at $p < 0.05$. Post Hoc comparisons of least square means of the fixed effects were conducted using Tukey's t-test.

Results

The different growth stages consistently affected sorghum forages' nutrient parameters and digestibility, while the sorghum type (non-*bmr* vs. *bmr*) did not affect most nutrient value parameters, including ADL (Table 1). There were no interactions between the sorghum growth stage and the sorghum type for the nutrient parameters and digestibility. The pairwise comparisons indicated higher CP in pre-boot stage sorghums and *bmr* type (Table 1). The post-hoc comparison of mean values did not differ in ADF and NDF even after the significance of the growth stage effect. The ADL of sorghum differed only by growth stage, but no difference was found between *bmr* and non-*bmr* sorghum ($p=0.19$). The IVTD of the pre-boot stage was greater than the half-bloom stage by 8.4% unit. No difference in IVTD was found between non-*bmr* and *bmr*.

Figure 1. presents ruminal gas production from non-*bmr* sorghum biomass and *bmr* sorghum and sudangrass, indicating the different ruminal fermentation patterns between the sorghum types. The non-*bmr* sorghum presented a dual plateau with two slopes of gas production, while the *bmr* type presented a single plateau. The final gas production volume ranged from 30 to 50 mL/g.

Table 1. Pairwise comparison by growth stage and sorghum type (non-*bmr* vs. *bmr*) for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent insoluble lignin (ADL), and *in vitro* true digestibility (IVTD).

Effect	Nutrient value parameter				
	CP	ADF	NDF	ADL	IVTD
Stage					
Mid-bloom	8.3b†	37.5a	61.6a	3.31a	67.4b
Pre-boot	15.3a	36.4a	59.7a	2.46b	75.8a
Sorghum Type					
Non- <i>bmr</i>	10.1b	36.4a	61.1a	2.85a	70.4a
<i>bmr</i>	13.5a	37.5a	60.2a	2.92a	72.9a

†, numbers followed by the same letter did not differ within the same effect at $p > 0.05$.

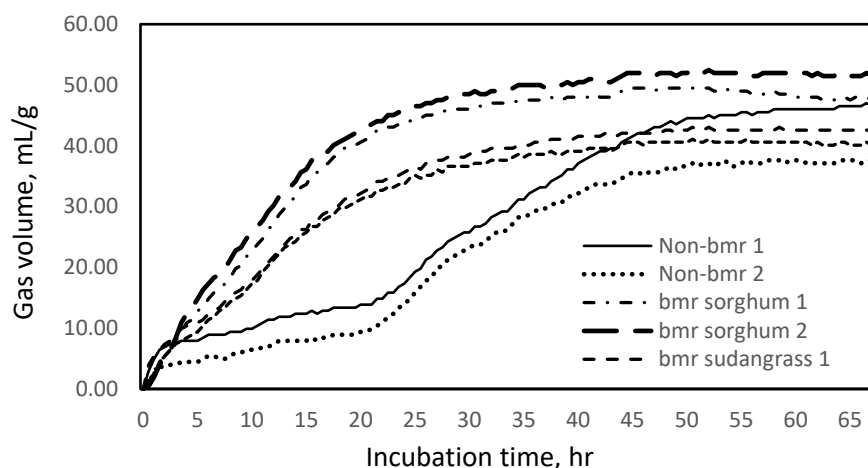


Figure 1. *In vitro* ruminal gas production of pre-boot growth stage non-*bmr*, *bmr* sorghum, and *bmr* sudangrass.

Conclusions

There were no significant differences in lignin content and 48-hour ruminal digestibility between the two sorghum forage types at the same growth stage, suggesting that optimizing the growth stage for sorghum harvest has a greater impact. While the numerical differences in digestibility are insignificant, faster ruminal fermentation of *bmr*-type sorghum may indicate faster rumen clearance with rapid degradation, leading to higher intake potential.

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Corn Hybrid Resistance Screening for Deoxynivalenol in Silage and Grain

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Keywords: Deoxynivalenol, Mycotoxin, Hybrid resistance, corn silage.

Introduction

Mycotoxins in feed are a major challenge to the world livestock industry. Deoxynivalenol, DON, is one of several mycotoxins produced as a secondary metabolite by the fungus *Fusarium graminearum*, which can cause ear and stalk rot in corn. DON is often also known as vomitoxin as it can cause vomiting in monogastric at low levels. Ruminates can detoxify some DON consumed in their feed. However, total DON levels in high-producing dairy cow rations may still need to be less than 1 ppm to decrease the negative effects of DON on reproductive performance and immune system suppression. The Great Lakes region in the United States has been seeing increased incidence of DON contamination going from a once every ten years probably in the area to a yearly challenge in at least a small part of the region. The fungus *Fusarium graminearum* has many hosts including small grains causing Fusarium Head blight, leading to head scab. In small grains disease, nurseries often called scab evaluation nurseries are used to rate varieties on their ability to resist infection from *Fusarium graminearum* and the production of DON. Since small grain varieties have genetic differences that make them more resistant or susceptible we establish a hybrid screen program in corn to evaluate large quantities of hybrids for their genetic susceptibility to DON production.

Materials and Methods

To get a wide representation of corn hybrid genetics we created a free corn hybrid screen program open to all seed companies who sold corn seed in Ohio. The requirements for entry of a hybrid into this screening were that it was a currently available hybrid and that all results would be published. Seed companies had the opportunity to enter hybrids into a corn grain-only screening or a corn silage screening. For both screening programs, an entry form was sent to seed companies through the Ohio Corn Performance Trial asking what hybrids they would like to enter and requesting seed by the First of May for planting in the screening trial. Both screenings included an inoculation of *Fusarium graminearum* spores during the green silk growth stage. To allow for all-natural mechanisms of disease resistance such as silk channel resistance inoculum was sprayed from a backpack sprayer onto the silks.

One location the Ohio State Northwest research station was used for the corn silage hybrid resistance screening while three locations were used for the corn grain hybrid resistance screening. For corn silage, a randomized complete block split block design was used. The blocks were split into hybrid maturity into early, middle, and late seasons to facilitate more efficient inoculation and harvest. Four-row plots were used with three replications. The center two rows were inoculated with *Fusarium graminearum* during the green silk growth stage. The two inoculated rows in the center were harvested with a Kemper silage harvester. Plot weights were taken along with a one-gallon silage sample. The silage samples were vacuum-sealed to ferment for 30 days and then analyzed for dry matter and DON contamination levels using High-performance liquid chromatography-mass spectrometry.

The grain screening was conducted in three locations across Ohio Wooster, South Charleston, and Bucyrus increasing the chances of favorable environmental conditions for disease development. The grain screening was a randomized complete block split-block split-plot design with only two replications at each location. The blocks were split by hybrid maturity of early, middle, and late seasons to allow for inoculation and more uniform pollination of the plots. Two row plots were used with one row being inoculated and the other uninoculated for natural infection. The inoculated row was paired next to the inoculated row in the adjacent plot so that during inoculation the over-spray would land on another inoculated plot and not the naturally infected plot. Single-row plots were harvested with an Almaco plot combine with a cyclone grain sampler. Grain samples were dried to below 15 percent moisture then ground and analyzed for DON concentration using High-performance liquid chromatography-mass spectrometry.

Results

Corn hybrids respond significantly differently when challenged by *Fusarium graminearum* fungus in the amount of stalk or ear rot and the amount of the DON mycotoxin produced. A hybrid screening program for DON susceptibility and partial resistance can help producers select corn hybrids with potentially lower DON levels. In 2023 the 3-location average of 78 hybrids had 43 hybrids with an average DON level below 2 ppm, 26 hybrids between 2-7 ppm DON, and another 9 hybrids greater than 7 ppm DON. Location was a significant factor in this trial. Each location experienced different weather conditions, with the locations that spent more time during pollination in the disease development window of 14.5-30°F with relative humidities greater than 80%. Treatments also included looking at each hybrid under both natural infection only or inoculation to increase fungal spores present. Under favorable weather conditions for disease development inoculation did not have a significant effect on hybrid DON levels.

The silage trial in 2024 had 11 hybrids successfully harvested, 10 more were submitted but lost due to flooding damage early in the season. Three hybrids had corn silage DON levels greater than 1 ppm and 8 hybrids less than 0.70 ppm DON with a C.V. of 61 and an LSD of 0.65, $P > 0.01$. The lowest hybrid only averaged 0.06 ppm DON while the highest hybrid averaged 1.46 ppm DON. While these hybrids were all from one company, the DON levels were

significantly different. Screening corn silage hybrids from all companies will allow producers to select hybrids that meet the levels of DON that are acceptable for their feed needs.

The corn grain susceptibility screening trial in 2024 had 89 hybrids submitted and harvested at 3 locations. Each location contained an inoculated and a natural infection treatment for each hybrid. Weather conditions in 2024 were unfavorable for fungal disease development at any of the 3 locations. Under unfavorable weather conditions for disease development, inoculation to increase fungal spores significantly affects DON levels. DON levels were much lower in 2024 with hybrid ranging from 0 ppm to 3.65 ppm at the location with the highest DON levels. Only 2 of the 3 locations had DON levels that, were significantly different between hybrids. The location with the greatest DON levels had 18 hybrids that were significantly higher than zero ppm DON at $P > 0.10$. By using more than one location even when all locations had low DON hybrids the most susceptible hybrids can still be detected.

Conclusion

Hybrid screening for DON resistance can be a critical component of the management of DON in silage. Weather conditions during pollination play a critical role in the DON levels at harvest even if the plots are inoculated so that fungal spores are present. Even under unfavorable weather conditions, significant differences can be detected between corn hybrids in both corn silage and corn grain at $P > 0.01$. The corn silage evaluation from 2024 had a hybrid DON range of 0.06 to 1.46 ppm. While the grain screen ranged from 0-3.45 ppm. A corn hybrid screen program that includes both corn grain and corn silage will be a critical tool to allow farmers to select hybrids with partial resistance. Since both the corn stalk and ear can be infected by *Fusarium Graminearum* a silage screen program will be better than a grain-only screen program for silage producers. However, selecting hybrids with grain resistance allows producers to have resistance from at least one of the two routes of DON production.

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Bacteriocins in Silage Management: A Natural Solution to Spore-Forming Bacteria Challenges

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Keywords: Silage fermentation, Bacteriocins, *Clostridium*, Microbiota, Spoilage

Introduction

Contamination of silage by *Clostridium* causes unfavourable silage fermentation, spoiling, and financial losses in the dairy industry (Driehuis et al., 2018). Through silage making and preservation, the heat-resistant *Clostridium* spores continue to exist, causing silage quality deterioration and subsequent problems in cheese production (Avila et al, 2020; le Bourhis et al., 2007). Generally Recognized as Safe (GRAS) natural antimicrobial peptides called bacteriocins are becoming increasingly popular as efficient substitutes for chemical preservatives. By targeting particular bacterial populations, these bioactive chemicals prevent or eliminate undesirable microorganisms while preserving product quality (Soltani et al, 2021). The objective of this study is to determine the efficacy of three bacteriocins (Nisin, Enterocin, and Brevibacillin) as well as a combination of Nisin and Brevibacillin in reducing *Clostridium* population in silage and their impact on silage quality and nutritive value.

Materials and methods

Silage was prepared using third-cut alfalfa at the 25% bloom stage. Forage was treated to produce silage liable to clostridial fermentation (moisture content higher than recommendation, ensiled at low density, contaminated with soil). The experiment was conducted in a randomized block design, with 4 experimental units per treatment. Forage was treated with saline solution (0.9% NaCl control), inoculated with clostridia, and then treated with bacteriocins (Ismail Fliss and Eric Biron research laboratories, Dep. Food Science, Université Laval, Qc, Canada). Treatments (10 in total) were control (no bacteriocin), Nisin, Enterocin, Brevibacillin, and Nisin + Brevibacillin, without or with clostridia inoculation. Except for negative control, a consortia of clostridia inoculum (5 clostridial strains) was applied to the forage (4kg fresh material, FM) at 5 log CFU/g (FM). Bacteriocins were applied at specified concentrations: Nisin (60 µg/g FM), Enterocin (15 µg/g FM), Brevibacillin (25 µg/g FM), and Nisin + Brevibacillin (7 µg/g FM). Forage was ensiled in experimental silos (1.7 L) in duplicates (for each sampling day), and was sampled immediately before ensiling (Day 0) and 4, and 100 days post ensiling (Day 4 and Day 100, respectively). Silage microbial population was determined by microbial count (total count, lactic acid bacteria (LAB), and *Clostridium*) and silage fermentation quality (lactic, acetic, and butyric acids) was quantified. Statistical analysis was conducted to determine treatment effects, using a two-way analysis of variance. The significance level was declared at $P < 0.05$ using Dennett's test.

Results

At Day 0, total bacterial and LAB (lactic acid bacteria) counts were similar across all treatments, averaging 8.0 log CFU/g and 5.0 log CFU/g, respectively. By Day 4, clostridia count in the untreated control silage averaged 6.4 log CFU/g. Treatments with Nisin and Nisin + Brevibacillin demonstrated significant reductions compared to the control, with clostridia counts reduced to 1.32 and 1.04 log CFU/g, respectively. Furthermore, clostridia were undetectable in silages treated with Brevibacillin and Enterocin (Table 1). On the other hand, the LAB population on Day 4 showed no significant differences across all treatments, indicating that bacteriocins did not negatively impact the LAB population. By Day 100, clostridia count in the untreated control silage remained elevated at 5.33 log CFU/g. In contrast, clostridia were completely undetectable in all silages treated with bacteriocins. Also, in silages inoculated with clostridia inoculum, bacteriocin treatments significantly suppressed clostridia growth compared to the clostridia-contaminated control. Enterocin treatment showed the lowest remaining clostridia count of 3.5 log CFU/g. LAB populations at Day 100 were greater in all bacteriocin-treated silages compared to both the untreated control and the clostridia-contaminated control. Organic acid analysis revealed improved fermentation profiles in silages treated with bacteriocins. By Day 4, lactic acid concentrations in untreated silages (control) were 1.6 g/kg DM, and the silages treated with Nisin demonstrated a greater amount of lactic acid at 2.2 g/kg DM. At Day 100, and in clostridia-inoculated silages, lactic acid concentration in bacteriocin-treated silages ranged from 1.2 g/kg DM for Brevibacillin-treated silage to 2.1g/kg DM in Nisin-treated silage, while it was 0.3 g/kg DM in silage without bacteriocin treatment. Acetic acid concentrations on Day 4 showed no significant differences among bacteriocin-treated silages compared to control apart from the treatment which was inoculated with clostridia and Brevibacillin where the amount reached 1.6 g/kg DM, compared to just 0.46 g/kg DM in untreated clostridia-inoculated silages. Butyric acid, a spoilage indicator, was significantly lower in bacteriocin-treated silages. By Day 4, butyric acid concentrations in clostridia-inoculated untreated silages reached 0.7 mg/kg DM, while bacteriocin-treated silages showed significantly reduced levels, with Brevibacillin-treated silage at 0.4 mg/kg DM. Similarly, in silages treated without clostridia inoculation, Brevibacillin-treated silage achieved a butyric acid concentration of 0.29 mg/kg DM compared to the untreated control at 0.34 mg/kg DM. By Day 100, treatments with Enterocin and the combination of Nisin + Brevibacillin achieved the highest lactic acid concentrations (nearly 4 g/kg DM), indicating enhanced fermentation efficiency and superior preservation quality. Among clostridia-contaminated treatments, the silages treated with Nisin showed the highest concentration of lactic acid at 2.3 g/kg DM compared to the clostridia-inoculated silages. The effects of bacteriocins were consistent in silages both inoculated and uninoculated with clostridia, demonstrating significant reductions in spoilage-associated metabolites and improvements in silage quality.

Conclusions

In conclusion, bacteriocin treatments significantly reduced clostridia counts, with some treatments rendering clostridia undetectable by Day 100, while simultaneously preserving or enhancing the LAB population and improving fermentation profiles by lowering spoilage-associated metabolites such as butyric acid. The results highlight that all bacteriocins performed effectively in reducing spoilage indicators and supporting lactic acid production. These findings underscore the potential of bacteriocins as sustainable biopreservatives to mitigate silage spoilage and improve feed quality. Future work should focus on optimizing dosage and application under field conditions.

Table 1. Effects of Bacteriocin treatments on clostridia counts (log CFU/g) in silage at Day 0, Day 4, and Day 100.

Treatments	Clostridia Counts			SEM
	Day 0	Day 4	Day 100	
Control	5.96	6.39	5.33	0.084
Nisin	2.11	1.32*	0.00*	0.140
Enterocin	1.76	0.00*	0.00*	0.054
Brevibacillin	1.95	0.00*	0.00*	0.011
Nisin+Brevibacillin	2.38	1.04*	0.00*	0.034
Clostridia	6.91	7.95	6.80	0.042
Clostridia+Nisin	3.22	4.06*	4.33*	0.158
Clostridia+Enterocin	2.53	2.03*	3.50*	0.304
Clostridia+Brevibacillin	2.09	4.32*	5.26*	0.162
Clostridia+Nisin+Brevibacillin	2.75	2.04*	5.74*	0.226
* indicates significant differences compared to the untreated control at the same time point and clostridia-contaminated silage at the same time point ($P < 0.05$). SEM: standard error of means				

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Meta Study on Preserving Grass Silage During Storage and Feed Out with SiloSolve FC

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Keywords: silage, inoculant, grass, varying dry matter, DM loss, aerobic stability

Introduction

The dry matter (DM) content of freshly harvested grass for silage is a critical factor influencing the fermentation quality and overall nutritional value of the silage produced. Optimal DM content for good quality silage typically ranges from 30% to 40% (Khota et al., 2018). As larger farm operations often harvest over a longer time period the forage dry matter is not always in the ideal range and might be too wet or too dry. The fermentation quality of silage is also affected by the harvesting and storage techniques employed. Factors such as mowing, wilting, chopping, and the use of microbial inoculants can significantly influence the DM content and the overall fermentation process (Randby et al., 2012). Silage inoculants enhance the fermentation process and improve stability during feed-out (Muck et al., 2018). However, it is important to note that the effectiveness of inoculants can vary based on several factors, including the specific strains used, the type of forage, and the environmental conditions during ensiling. Consequently, some farmers might choose not to use a silage inoculant, resulting in increased dry matter losses and diminished economic returns. This meta-study aims to compile results from six grass silage trials conducted over the past decade, testing a combination of *Lactococcus lactis* and *Lentilactobacillus buchneri* (SiloSolve® FC, Novonesis), with a focus on fresh and dry matter loss and the aerobic stability of grass at various dry matter.

Materials and methods

For the meta-analysis grass mixtures without legumes (e.g. alfalfa or clover) from six harvest times were collected. The harvest took place at three different sites in Denmark, Lithuania, or Germany from the harvest years 2019-2023. Only trials, where at least fresh matter loss and aerobic stability amongst many other parameters were reported, were included in the meta-analysis. Some studies additionally included different wilting periods (0 to 2 days) whereby the influence of different dry matter content on the same forage was tested. Additionally, the effect of different anaerobic fermentation time was tested (7 to 90 days). The grass was either inoculated with 1.5×10^5 CFU/g of SiloSolve® FC (Novonesis, Lyngby, Denmark) or without any inoculant (control). The SiloSolve® FC contains two bacterial strains *Lactococcus lactis* (DSM11037) and *Lentilactobacillus buchneri* (DSM22501) applied at 150,000 CFU/g of fresh matter in all studies. After the anaerobic storage, chemical analysis and microbial count were conducted, except from one study where dry matter was measured, but no further chemical analysis was carried out.

The silage was stored either in mini silos of up to 1 kg or in big bales (over 600 kg bales) with three to five replicates per treatment. The aerobic challenge was done in mini silo scale of less than one kg, while one study was done in both big bale and mini silo. The ambient temperature during aerobic challenge was 20–25 °C but stable within each trial ($\pm 1^\circ\text{C}$), except from the big bale trial which followed the outdoor temperature. Temperature dataloggers were placed in the middle of the silage and the aerobic challenge lasted up to 30 days. Aerobic stability was defined as the duration (in hours) for which the silage remained stable, i.e., before the temperature rose 3°C or more above ambient temperature.

Each harvest time, wilting and anaerobic storage time served as the experimental unit for statistical analysis with inoculation as fixed effect.

Results

Due to wilting for 0 to 2 days, the dry matter content varied between 20.8% and 72.6%. The inoculation of SiloSolve® FC reduced fresh matter loss during anaerobic storage, but only to a small extent. Compared with the non-inoculated control, one study showed a significant difference during aerobic challenge: after two days of wilting, followed by 70 days of anaerobic storage and 30 days of aerobic challenge, the control experienced a fresh matter loss of 18.4%, while the inoculated silage lost only 4.2%.

Aerobic stability was highly dependent on the forage dry matter. The average aerobic stability of the non-inoculated control was 181 hours, with a range from 19 hours to 679 hours. In contrast, inoculated grass silage demonstrated increased stability, averaging 251 hours before the temperature rose above 3°C from ambient temperature. The minimum time recorded was 27 hours, while the maximum reached 710 hours.

Table 1. Dry matter loss during anaerobe storage, aerobic challenge and the time of aerobic stability of grass silages inoculated with and without SiloSolve FC from six harvest, wilting periods and anaerobe storage time.

	Inoculant	Minimum	Maximum	Average
Dry matter of forage used for inoculation trial (%)		20.8	72.6	37.2
Crude protein of forage used for inoculation trial (% of DM)		9.1	19.7	14.6
Fresh matter loss (%) during anaerobe storage*	Control	0.2	2.1	1.2
	SiloSolve FC	0.1	2.1	1.2
Fresh matter loss (%) during aerobe challenge*	Control	2.2	18.4	6.7
	SiloSolve FC	1.6	9.4	3.8
Aerobic Stability* (h until 3 °C above ambient temp.)	Control	19	679	181
	SiloSolve FC	27	710	251

*Where anaerobe storage could be between 7 and 90 days and forage wilted in 0, 1 or 2 days.

Conclusions

The inoculation of SiloSolve FC containing the strain combination of *Lactococcus lactis* (DSM11037) and *Lentilactobacillus buchneri* (DSM22501) reduced fresh matter loss and improved aerobic stability across different dry matter content and storage time of grass silage, demonstrating the versatility of the silage inoculant.

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Inoculant and Dry Matter Effects on the Aerobic Stability and Nutrient Degradability of Corn Silage

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Keywords: *Lentilactobacillus buchneri*, *Lentilactobacillus hilgardii*, starch degradability

Introduction

Silage inoculants combining facultative and obligate heterofermentative lactic acid bacteria (LAB) are primarily applied to optimize fermentation and enhance aerobic stability (AS) (Muck et al., 2018). Beyond these primary functions, LAB inoculants have improved nutrient degradability under certain conditions (Muck et al., 2018). Factors such as dry matter (DM) content and ensiling duration may influence fermentation, AS, and nutrient degradability. To explore these effects, two experiments were conducted to evaluate the influence of an inoculant on the fermentation characteristics, nutrient degradability, and AS of whole-plant corn silage. These studies assessed variations in ensiling durations (Experiments 1 and 2) and DM levels (Experiment 2).

Materials and methods

In 2021, a brown midrib corn variety was harvested at 35.44% DM for Experiment 1. In 2022, a conventional corn variety was harvested at two DM levels: low (26.18%) and high (39.68%). Whole corn plants were either untreated (CTRL) or treated with an inoculant (INO) containing *Lentilactobacillus hilgardii* CNCM-I-4785 (150,000 cfu/g of fresh forage), *Lentilactobacillus buchneri* NCIMB 40788 (150,000 cfu/g of fresh forage), *Pediococcus pentosaceus* NCIMB 12455 (100,000 cfu/g of fresh forage), beta-glucanase (5,750 IU/g), and xylanase (30,000 IU/g). Forage was packed into 7.57-L silos at densities of approximately 220 kg of DM/m³ (Experiment 1) and 230 kg of DM/m³ (Experiment 2) and stored at 21 ± 2°C for 14 and 90 d (Experiment 1) or 30, 60, 120, and 180 d (Experiment 2).

The analyses included measurements of pH, concentrations of lactic acid, acetic acid, 1,2-propanediol, and prolamin as well as *in vitro* organic matter degradability (OMD) at 48 h, NDF degradability (NDFD) at 12 and 30 h, and starch degradability (starchD) at 7 h, counts of LAB and yeast, and AS. Aerobic stability was measured as the time (h) until silage temperature increased by 2°C following air exposure at 21 ± 2°C. Data analysis was performed using R (version 4.2.3). For Experiment 1, the Kruskal-Wallis test was used to analyze the data. In Experiment 2, a 2 × 2 factorial design (inoculation and DM level) was employed and analyzed using two-way ANOVA, followed by Tukey's post-hoc test for comparisons with $P \leq 0.050$. Statistical significance was defined as $P \leq 0.050$.

Results

In Experiment 1, INO had higher LAB counts (log₁₀ cfu/g silage) than CTRL at 14 d (8.78 vs. 7.97) and 90 d (8.96 vs. 6.24). Similarly, in Experiment 2, INO showed greater LAB counts than CTRL across both DM levels at 30 d (average of 8.73 vs. 7.35) and 60 d (average of 9.00 vs. 6.75, on average). At 120 d, INO also had higher LAB counts than CTRL in both low and high DM silages, with a more pronounced difference in low DM (8.99 vs. 7.21) than in high DM (8.91 vs. 8.00) silage. At 180 d, the CTRL low DM treatment had the lowest LAB count (7.26 log₁₀ cfu/g silage), whereas the other treatments averaged 8.45 log₁₀ cfu/g silage.

In Experiment 1, inoculation did not affect pH and lactic acid content, which was unexpected. Typically, inoculation with obligate heterofermentative LAB leads to the conversion of the stronger acid, lactic acid, into the weaker acid, acetic acid, resulting in a pH increase (Kung et al., 2018). In Experiment 2, the results aligned more closely with this expected pattern, except in low DM silage at 30 d, where INO had a lower pH than CTRL (3.87 vs. 3.89). Conversely, in high DM silage at 30 d, INO had higher pH than CTRL (3.96 vs. 3.93). At 120 d, INO had a higher pH compared to CTRL in both low (3.81 vs. 3.69) and high DM silages (4.01 vs. 3.95), with the difference being more pronounced in low DM silage. Additionally, INO had a higher pH than CTRL at 60 d (average of 3.89 vs. 4.01) and 180 d (average of 3.90 vs. 3.77) across both DM levels. Inoculation did not impact lactic acid content at 30 d in either DM levels or at 60 and 120 d in high DM silages. However, in low DM silages, INO had lower lactic acid content than CTRL at 60 d (5.43 vs. 6.70% DM) and 120 d (7.12 vs. 9.08% DM). Furthermore, at 180 d, INO had lower lactic acid content than CTRL across both DM levels (average of 5.68 vs. 7.40% DM).

Both *L. buchneri* and *L. hilgardii* can convert lactic acid into acetic acid and 1,2-propanediol (Muck et al., 2018), making an increase in these compounds expected in INO silages. In Experiment 1, INO had higher acetic acid levels (% of DM) than CTRL at 14 d (1.41 vs. 0.98) and 90 d (2.41 vs. 1.26). Similarly, 1,2-propanediol levels (% DM) were higher in INO than CTRL at 14 d (0.28 vs. < 0.01) and 90 d (1.20 vs. 0.08). In Experiment 2, at 30 d, INO had higher acetic acid content than CTRL in low DM silage (1.89 vs. 1.49% DM). However, inoculation had no effect in high DM silage at 30 d. At 180 d, inoculation increased more substantially the acetic acid content (% DM) in low DM (INO = 4.57 and CTRL = 2.20) than in high DM silage (INO = 2.50 and CTRL = 1.37). Across both DM levels, INO had more acetic acid (% DM) than CTRL at 60 d (average of 1.89 vs. 1.20) and 120 d (average of 3.12 vs. 1.40). Furthermore, INO had higher 1,2-propanediol levels than CTRL across all ensiling durations and DM levels. The increases in 1,2-propanediol (% DM) in INO compared to CTRL were more pronounced in low DM (30 d: 0.26 vs. < 0.01; 60 d: 0.70 vs. < 0.01; 120 d: 2.01 vs. 0.07; 180 d: 2.62 vs. 0.27) than in high DM silages (30 d: 0.22 vs. < 0.01; 60 d: 0.51 vs. < 0.01; 120 d: 1.52 vs. 0.07; 180 d: 1.67 vs. 0.26).

In Experiment 1, inoculation did not affect yeast counts at 14 d. However, at 90 d, INO had fewer yeasts than CTRL (< 2.00 vs. 4.85 log₁₀ cfu/g silage). In Experiment 2, yeast counts were not evaluated at 30 d but were consistently below the detection limit (< 2.00 log₁₀ cfu/g silage) in INO at 60, 120, and 180 d for both DM levels. At 60 d, yeast counts in CTRL were higher in low DM than in high DM (5.55 vs. 4.94 log₁₀ cfu/g silage). At 120 d, CTRL had yeast counts of 4.62 and 5.09 log₁₀ cfu/g silage for low and high DM, respectively, and at 180 days, yeast counts in CTRL were 3.56 and 4.37 log₁₀ cfu/g silage for low and high DM, respectively. Yeasts are the primary initiators of aerobic deterioration in corn silage, and their suppression by inoculants enhances AS (Muck et al., 2018). In Experiment 1, INO had slightly higher AS than CTRL at 14 d (32 vs. 29 h) but showed markedly higher AS at 90 d (400 vs. 40 h). In Experiment 2, INO had higher AS (h) than CTRL at 30 d (61 vs. 31), 60 d (77 vs. 30), and 180 d (115 vs. 27). At 60 d, the difference in AS (h) between INO and CTRL was more pronounced in low DM (88 vs. 30) than high DM (66 vs. 30). At 120 d, INO had higher AS than CTRL in low DM silage (189 vs. 27 h), whereas inoculation did not significantly affect AS in high DM silage (INO = 84 h and CTRL = 25 h).

In Experiment 1, inoculation had no effect on starchD at 14 d, and prolamin, NDFD, and OMD were not evaluated at this time. At 90 d, inoculation did not affect prolamin levels, but INO had higher starchD (56.7 vs. 46.9% starch) and 30-h NDFD (60.3 vs. 57.4% NDFD) compared to CTRL, but OMD was unaffected by inoculation. In Experiment 2, prolamin content was affected by inoculation, with the greatest difference observed at 180 d (CTRL = 3.4 and INO = 0.8% DM). In Experiment 2, at 60 d, INO had lower starchD than CTRL in high DM silage (65.4 vs. 68.5% starch) but higher starchD than CTRL in low DM silage (83.2 vs. 82.1% starch). Moreover, INO had a higher starchD (% starch) than CTRL at 120 d (81.8 vs. 77.1) and 180 d (80.2 vs. 77.5). Inoculation did not affect 12-h NDFD for either DM level at 60 and 120 d, nor for low DM silages at 30 and 180 d. Similarly, 30-h NDFD was unaffected by inoculation for both DM levels at 60, 120, and 180 d. However, in high DM silage, INO had higher 12-h NDFD (% NDF) than CTRL at 30 (29.3 vs. 25.5) and 180 d (31.1 vs. 26.3). Additionally, INO improved 30-h NDFD compared to CTRL at 30 d (52.6 vs. 51.2% NDF). INO also had higher OMD (% OM) than CTRL at 30 d (74.8 vs. 74.3), 120 d (75.1 vs. 74.9), and 180 d (75.9 vs. 75.3).

Conclusions

Inoculation increased acetic acid content, inhibiting yeast growth and enhancing AS of corn silage in both experiments. The inoculant improved AS in both low and high DM silages as early as 30 d post-ensiling, with a more pronounced effect in low DM compared to high DM at 60 and 120 d. Whereas inoculation did not consistently affect nutrient degradability, it enhanced starchD after long-term storage (> 90 d) in both experiments, and improved NDFD and OMD under specific conditions in Experiment 2.

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Can Giant-reed Silage Be an Alternative Forage Source for Heifers and Beef-cattle in an Arid Area?

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Keywords: giant-reed, *Arundo donax*., silage, ruminant, on-farm trial

Introduction

Due to the droughts in recent years, Hungarian researchers and farmers are in need of finding new plants to cultivate for ruminants, that are capable of growing in an ever-warming climate. The aim of this study was to research whether giant-reed (*Arundo donax* L.) would be ideal as a supplementation. This perennial grass is used globally for bioethanol, biogas and biomass. It is globally used for the fitoremediation of contaminated soils (Fernando et al. 2016). In the temperate continental climate of Hungary its seeds are sterile, therefore it is not an invasive species. It produces an average yield even in a year of drought and heat-stress and tolerates periodic aridity (Simon 2017). The reaping of giant-reed can be easily mechanized, the potential dry matter content is 25-30%. Single harvest technology is preferable in the matter of a better crude ash content, adequate crude protein content and sufficient sugar content for fermentation.

Materials and methods

The harvested giant-reed was planted in 2018, several laboratory trials were run in 2021, 2022 and 2023. In 2022 the plant was harvested for a model ensiling test (Trial 1) and in 2023 an on-farm harvesting and feeding trial took place (Trial 2).

Trial 1: In the laboratory trials, the giant-reed was cut by hand in October at height of 1,6 m and was taken to the laboratory for chopping then packed in buckets for fermenting. The samples were examined with near infrared (NIR) analysis. Measured parameters: dry matter, crude protein, crude fat, crude ash, sugar, starch, amylase-treated, ash-corrected neutral detergent fiber (aNDFom), acid detergent fiber (ADF), acid detergent lignin (ADL), non-fibrous carbohydrate (NFC), soluble crude protein, nitrate, organic matter digestibility 48-hour post incubation (OMd₄₈) and degradable NDF 48-hour post incubation (dNDF₄₈).

Trial 2: On June 24. 2023, giant-reed was harvested for an on-farm feeding trial. It was cut by traditional forage harvester, with an average height of approx. 2,0-2,2 m and 20 cm stubble. The average yield was 15 t AF/ha. The chopped forage was packed into a bunker silo and covered with plastic sheet. The ensiled material was opened and fed with heifers for four days in August 2023.

Results

In 2022 a model silage test was implemented to study the fermentability of giant-reed. Nutrient content and fermentational values are presented in tables 1-3. (Trial 1).

Table 1. Dry matter, crude protein, crude fat, crude fiber and crude ash contents of the giant-reed model silage (2022.10.05., Hungary)

Dry matter g/kg DM	Crude protein g/kg DM	Crude fat g/kg DM	Crude fiber g/kg DM	Crude ash g/kg DM
367	86	25	377	77

Table 2. NDF, ADF, ADL, OMd, NDFd₄₈, dNDF₄₈, NEI, sugar, soluble protein (% and g/kg DM) and nitrate content of giant-reed model silage (2022.10.05., Hungary)

NDF g/kg DM	ADF g/kg DM	ADL g/kg DM	OMd g/kg DM	NDFd ₄₈ g/kg DM	dNDF ₄₈ g/kg DM	NEI g/kg DM	Sugar g/kg DM	Soluble protein %	Soluble protein g/kg DM	Nitrate g/kg DM
762	448	54	54	43,1	328	4,2	<12	88	76	2

Table 3. The pH, NH₃, lactic acid, acetic acid and lactic acid/acetic acid ratio (2022.10.05., Hungary)

pH	NH ₃ g/kg DM	Lactic acid g/kg DM	Acetic acid g/kg DM	Lactic acid/acetic acid
4,5	11	53	9	5,9

Based on the results, the conclusion is that giant-reed can be ensiled well and has a favorable fermentation profile in a model size. The nutrient content of the on-farm experiment are presented on tables 4-5. (Trial 2).

Table 4. Dry matter, crude protein, crude fat, crude fiber, crude ash and sugar contents of the on-farm giant-reed experiment (n=3, 2023.08.08., Hungary)

	Dry matter g/kg DM	Crude protein g/kg DM	Crude fat g/kg DM	Crude fiber g/kg DM	Crude ash g/kg DM	Sugar g/kg DM
Mean	223	91	22	403	106	12
Standard deviation	2,0	0,0	1,2	5,5	7,2	0,0
CV%	0,9	0,0	5,3	1,4	6,8	0,0
RMSEPy		5,43	2,56	9,61		6,91
Bias		-0,29	-0,09	-0,88		-0,59
R ²		0,99	0,89	0,98		0,97

RMSEPy - the actual prediction error on the validation dataset, BIAS- the average difference between predicted and actual value, R² –coefficient of determination

Table 5. NDF, ADF, ADL, OMd, NDFd₄₈ and dNDF₄₈ contents of the on-farm giant-reed experiment (n=3, 2023.08.08., Hungary)

	NDF g/kg DM	ADF g/kg DM	ADL g/kg DM	OMd %	NDFd ₄₈ %	dNDF ₄₈ g/kg DM
Mean	687	440	54	60	45	308
Standard deviation	5,1	4,4	1,0	0,8	1,3	11,3
CV%	0,7	1,0	1,9	1,3	2,9	3,7
RMSEPy	16,74	10,77	3,91			14,57
Bias	1,26	0,11	0,38			-0,31
R ²	0,97	0,97	0,94			0,93

RMSEPy - the actual prediction error on the validation dataset, BIAS- the average difference between predicted and actual value, R² –coefficient of determination

The TMR composition at the time was 3 kg AF/day/heifer triticale silage, 5 kg AF/day/heifer maize silage, 4 kg AF/day/heifer wet CGF, 3 kg AF/day/heifer hay and 2 kg AF/day/heifer grain-mix on the farm. 5 kg of giant-reed silage substituted the 3 kg AF of triticale silage for four days. During the feeding trial, the 114 heifers did not refuse the TMR containing a 5 kg AF reed silage/day. They did not sort out the giant-reed silage from the TMR, nor leave it.

Conclusions

We can conclude the giant-reed fermentation parameters can be adequate, if the harvest and ensiling technology was well managed. In addition, the on-farm feeding trial confirmed that giant-reed harvest is feasible, and dry matter intake of heifers will not be impaired by the reed silage (5 kg AF/da/heifer), if the NDFd₄₈ value is over 40% NDF. Further investigations are needed to find more details about the optimal growing stage at harvest and month of the year, weather.

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Impact of Dry Matter Losses on the Harvest Related Carbon Dioxide Emissions of Maize Silage

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Keywords: Maize silage, dry matter losses, carbon dioxide emissions

Introduction

In Europe dairy companies consider climate friendly milk production as an impact factor for their paying system. Harvesting maize is always related to a certain fuel consumption of the machinery used for chopping, transporting and packing of the maize crop. Dry matter (DM) losses between filling a silo and feed out are resulting in an increase of fuel demand related to harvested maize crop. Aim of this study was to show the importance of minimizing DM losses to reduce the harvest related carbon dioxide emissions.

Materials and methods

In two consecutive years (2023 and 2024) the DM yield of silage maize crop at the agricultural research and education center “Haus Riswick”, Kleve, Germany was determined by weighing every single trailer from each field on a gauged weighbridge. Dry matter content of the freshly harvested maize crop was determined by oven drying samples from every trailer of the individual fields. Figures for consumed diesel fuel were provided by the agricultural contractor and include the entire harvest work with chopping, transporting and packing of the maize crop. With a given average density of diesel fuel (833 g/Liter (l)) (DIN EN 590; 2022) and a carbon content of 86,32 % (Juhrich, 2022) a carbon weight of 719 g/l diesel fuel can be calculated. To oxidize 719 g of carbon 1915 g of oxygen are needed. Burning 1 liter of diesel fuel releases 2634 g of carbon dioxide (CO₂). To show the impact of DM losses on harvest related CO₂ emissions the figures of Koehler et al. (2013) were used. They report of DM losses in an “all in – all out” comparison for maize ensiled in bunkers in southern Germany ranging from nearly 0 up to 20% DM losses.

Results

The following Table 1 shows acreage, total DM yield, DM yield per hectare (ha) and the harvest related diesel fuel consume of the respective year. Figures show the accumulated values for at least three harvest dates. A direct comparison of the two consecutive years among themselves is inopportune as not only weather conditions but also fields differed and with them transport distances and number of transport vehicles.

Table 1: acreage, yield and diesel fuel consume

	2023	2024
acreage of maize crop in ha	47,0	56,3
total yield in t DM	803,04	867,21
yield t DM /ha	17,09 ±1,4	15,39 ±1,9
consumed diesel fuel in l	6619	7014
l diesel fuel /ha	140,83 ±9,8	124,50 ±10,9
l diesel fuel /t DM	8,24 ±0,4	8,09 ±0,4

Table 2 combines the work of Koehler et al. for on farm height of DM losses with realized figures for DM yield for maize crop. Further on it shows the impact of increasing DM losses on diesel fuel demand for the year 2023.

Table 3 shows figures for the second analyzed year 2024.

In both years a comparable diesel fuel consume for maize harvest with slightly more than 8 liters per t DM was realized.

With increasing losses and resulting decreasing remaining dry matter the relative diesel fuel consume per ton dry matter increases and with this the harvest related CO₂ emissions.

Table 2: impact of DM losses on diesel fuel demand and related CO₂ emissions (year 2023)

DM losses in %	remaining t DM	l diesel fuel / t DM	kg CO ₂ / t DM	increase of released CO ₂ in %
0%	803,04	8,24	21,71	0,0
5%	762,89	8,68	22,85	5,3
10%	722,74	9,16	24,12	11,1
15%	682,58	9,70	25,54	17,6
20%	642,43	10,30	27,14	25,0

Table 3: impact of DM losses on diesel fuel demand and related CO₂ emissions (year 2024)

DM losses in %	remaining t DM	l diesel fuel / t DM	kg CO ₂ / t DM	increase of released CO ₂ in %
0%	867,21	8,09	21,30	0,0
5%	823,85	8,51	22,43	5,3
10%	780,49	8,99	23,67	11,1
15%	737,13	9,52	25,06	17,6
20%	693,77	10,11	26,63	25,0

Conclusions

The observed years 2023 and 2024 showed that in years without yield affecting droughts a comparable amount of diesel fuel of about 8l is needed to get one ton of maize crop dry matter chopped, transported and packed. With a variation of fields, transport distances and weather conditions for the examined years those 8 liters seem to be a rather constant value for the maize harvest conditions at the research and education center in Kleve.

Minimizing losses is not only important for the quality of the ensiled feed and for the own economic benefit, reducing dry matter losses also help to minimize the unavoidable harvest related diesel fuel based CO₂ emissions and thus may help to fulfil dairy industry sustainability requirements.

Already available plant based diesel fuel substitutes can help to reduce harvest related CO₂ emissions remarkably as with their combustion only as much Carbon dioxide is released as plants have fixed before during photosynthesis.

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Review: DM losses of Grass and Other Forages from Harvest Through Conservation, Storage and Feedout to Intake

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Keywords: dry matter loss, fermentation failures, effluent, mould, spoilage

Introduction

According to Steinhöfel et al. (2023) forage conservation aims to preserve the nutrients quantitatively and qualitatively over a longer period by inactivating microbial and feed-specific enzymes, i.e. to limit the decomposition processes to an unavoidable or desirable extent. The preservation success is defined by the amount of material and mass losses and the change in feed value, fermentation quality and hygienic status. Depending on the type of preservation, the given environmental conditions and the degree to which the processes are controlled, there are varying degrees of quantitative and qualitative changes in the course of feed harvesting, preservation, storage and feed removal, particularly in the form of losses of energy and nutrients. Forage and feed management with all its related processes is therefore of central and immediate importance for the success on farm.

In addition to Borreani et al. (2018), this review article is intended to provide an up-to-date overview of dry matter (DM) losses within the feed production process chain. It also describes the magnitude of unavoidable and avoidable DM losses that may occur under favourable and unfavourable conditions, especially when extreme weather events such as heat, drought or heavy rainfall jeopardise forage production in terms of quantity and quality.

Material and Methods

According to Köhler et al. (2019), the basis for the evaluation of DM losses in animal feed is the gravimetric difference method (total-in vs. total-out). That means at least two weighings within an interval and the determination of the DM by means of oven or freeze drying (VDLUFA, 2012). Field losses are determined by the forage masses remaining on the ground after harvesting. These forage particles are collected and weighed from the soil either using a vacuum cleaner (Beckhoff et al. 1979) or plates with artificial stubble according to Lucas (2009). Silage losses were examined from ensiling to feed-out.

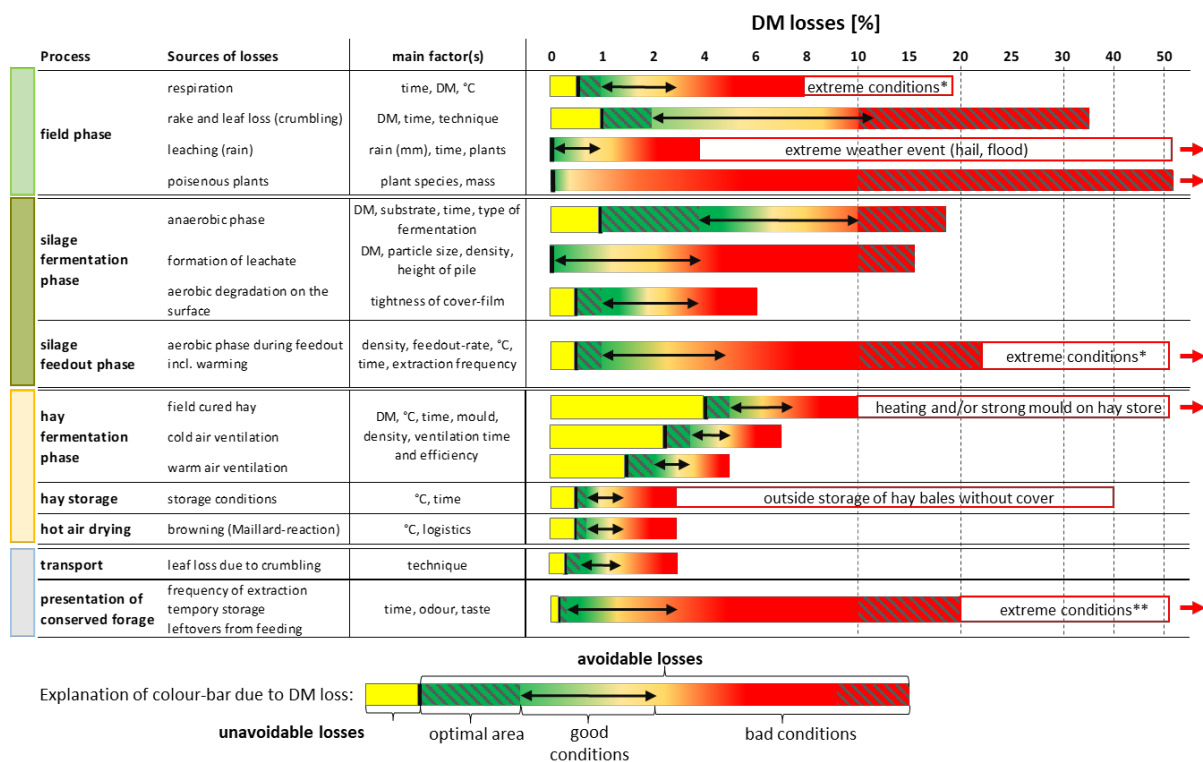
Results and Discussion

Mechanical DM losses range from 3 to 32% and depend on DM content, field retention time and weather conditions (Zimmer 1977). The level of ensiling losses is influenced by the DM content, the type and intensity of fermentation (lactic, acetic, butyric acid and alcoholic fermentation) and the energy consumption by the metabolic pathways of various microorganisms (Rooke & Hatfield, 2003). According to Resch & Buchgraber (2006), effluent losses can be up to approx. 15% DM. They are strongly related to the DM content, chop length, compaction and stack height in the clamp.

During hay storage, DM losses occur due to microbial degradation, which according to Rotz et al. (1991) can be estimated at 1 to 8% (Fig. 1).

Conclusions

In forage production, material DM losses are caused by a cascade of biotic and chemical processes that are influenced by environmental and management factors. In total, DM losses from plant growth (field) to feed intake (farm animal) for grass silage are around 10% under optimal conditions, 15 to 20% under average conditions and above 30% under unfavourable conditions. For field cured hay, DM losses below 16% would be very favourable, at approx. 25 to 30% average and at more than 40% of DM unfavourable. Knowledge of the causes of losses and their effects is complex, but of great importance for grassland and livestock farming.



Note on scaling: Leaps in numbers due to DM-losses are up to 10 % smaller for a better overview. ■ above 10 % leaps of numbers become larger.

*Extreme conditions: **Respiration**: wet-hot weather; **Silage-warming**: strong heating resp. rain entry and putrefaction in big silos without covering

**Extremes due to forage presentation: strong feed selection resp. refusal to feed; i.E. of ester-odour in silages

Fig. 1: Unavoidable and avoidable DM losses depending on the prevailing conditions and the processes in forage harvesting, preservation, storage, feedout and forage presentation

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Use of a Heterofermentative Inoculant in Mixed Crop Corn Silage

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Keywords: aerobic stability, corn silage, *Lactobacillus*, mixed crop silage, silage fermentation

Introduction

Corn silage is an excellent forage source for ruminants, but high regional concentration of corn acres has negative impact on the insect biodiversity in agricultural landscapes. One possible approach to improve the environmental impact of corn silage production, is the co-cultivation of corn together with flowering plants (e.g., legumes or sunflowers), that are more attractive to insects (Norris et al, 2018). In 2024, more than 98,000 ha mixed crop corn were grown in Germany (DMK, 2024). Aside from its positive impact on insect biodiversity, co-cultivation of corn together with legumes, such as beans, has the benefit of N fixation, potentially leading to a reduction in the need to fertilize N. The co-cultivation of corn and legumes might also lead to an increase of the low crude protein content of corn silage. However, the impact of ensiling corn mixed with other crops is not well researched. An earlier study (Benkner, 2020) has shown that the aerobic stability of corn silage is reduced, when ensiled together with increasing percentages of common beans. Commonly, heterofermentative silage inoculants are used in corn, to improve fermentation characteristics and aerobic stability. However, for mixed crop corn silages, there is a lack of data concerning overall silage quality and the use of inoculants. This study was designed, to compare the nutrient composition, fermentation quality, and aerobic stability of corn silage to that of mixed crop corn silage with field beans, common beans, and sunflowers and to evaluate the response of those silages to a heterofermentative silage inoculant.

Materials and methods

For this experiment, corn was planted alone or together with common beans (*Phaseolus vulgaris*, WAV612/Cinteo, Sativa Rheinau AG, Rheinau, Switzerland), field beans (*Vicia faba*, Fuego, NPZ/Saaten-Union, Holtsee, Germany) or sunflowers (*Helianthus annuus*, Lluís, R.A.G.T. Saaten GmbH, Hiddenhausen, Germany). The same variety corn (Geox Duo, R.A.G.T. Saaten GmbH, Hiddenhausen, Germany) was used in all four treatments (corn=C, corn+common beans=CCB, corn+field beans=CFB, corn+sunflowers=CSF). The crops were seeded on April 21st, 2021 in the same field in a small plot design, with four plots per treatment. The stands were harvested using a self-propelled small plot corn harvester (Haldrup GmbH, Ilshofen, Germany) on October 10th, 2021 (late dough stage of the corn). The forages were transferred to the lab, where one half of the material was inoculated with a mixture of heterofermentative lactic acid bacteria (*Lentilactobacillus buchneri* and *Lentilactobacillus diolivorans*; Lactosan GmbH & Co. KG, Kapfenberg, Austria) at 2.5×10^5 CFU/g FM (Cinoc, CCBinoc, CFBinoc, CSFinoc). To achieve even coverage, the inoculant was suspended in 5 ml tap water/kg FM. The other half of the material was sprayed with 5 ml tap water/kg FM, as control treatments (Ccon, CCBcon, CFBcon, CSFcon). The forages were manually packed into PVC mini silos (3.4 L total volume), and compressed three times with a pneumatic silage press (target packing density: 240 kg DM/m³). Once filled, the mini silos were sealed by a rubber lid held on by a metal clamp. Each lid had a gas relief valve, to allow release of excess fermentation gas. Six mini silos were filled per treatment. Three silos of each treatment (n=3) were opened 7d after ensiling, to measure the initial drop in pH. After 105d of ensiling, the remaining silos (n=3) were opened and sampled for chemical composition, pH, concentration of fermentation products and enumeration of LAB, yeasts, mold, and clostridia. The remaining silage was placed in insulated containers and stored at room temperature for 14d. Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY, USA) were placed in the mid-layer of the silage to record the heat loss from the silages as indicator for aerobic stability. Data were analyzed using the mixed model procedure of SAS (version 9.4). Substrate (C, CCB, CFB, CSF), inoculation with LAB and their interaction (substrate $\times$ inoculation) were considered as fixed effects. Individual mini silo was included as random effect. Differences between least squares means (LSM) were determined by the PDIF option using the Tukey's adjustment. Differences among treatments were declared significant at $P < 0.05$.

Results

Co-cultivation of corn with common beans (92.7 g/kg DM), field beans (71.7 g/kg DM), and sunflowers (88.3 g/kg DM) only led to moderate increases in crude protein (CP), compared to corn alone (65.2 g/kg DM; Table 1). Starch was by far the lowest for CSF (51 g/kg DM), since the sunflowers outcompeted the corn relatively early on in the growth season, leading to fewer and smaller cobs. CSF also had by far the highest buffering capacity (111 g lactic acid) followed by CCB (39.8 g lactic acid), CFB (26.4 g lactic acid), and C (22.9 g lactic acid; all /kg DM).

Table 8 Chemical composition and buffering capacity of the forages before ensiling (n=2).

Item	Treatment ¹			
	C ²	CCB	CFB	CSF
Dry matter, g/kg	413	335	397	306
Organic matter, g/kg DM	960	934	956	882
Crude protein, g/kg DM	65.2	92.7	71.7	88.3
Neutral detergent fiber ² , g/kg DM	419	442	415	442
Acid detergent fiber ³ , g/kg DM	241	286	235	394
Starch, g/kg DM	283	236	288	51
Sugar, g/kg DM	72.0	51.6	54.4	54.3
Buffering capacity, g LA ⁴ /kg DM	22.9	39.8	26.4	111

¹ C=corn, CCB= corn+common beans, CFB=corn+field beans, CSF=corn+sunflowers.

² Neutral detergent fiber was assayed with a heat-stable amylase and expressed without residual ash.

³ Acid detergent fiber expressed without residual ash.

⁴ Lactic acid.

Similarly to the fresh forages, the CP content of the silages differed across treatments ($P<0.001$), while inoculation had no effect on CP (data not shown). Driven by the very low starch content of CSF the NEL of CSF silage (CSFcon: 4.53, CSFinoc: 4.58 MJ NEL/kg DM) was about 2 MJ NEL/kg DM lower compared to all other treatments (6.34 to 6.96 MJ NEL/kg DM). In pure corn silage, inoculation led to a more rapid decline in pH (Cinoc: 0.15 unit/d) over the first 7d of ensiling, compared to Ccon (0.12 unit/d; $P<0.001$), whereas inoculation did not affect the initial pH decline in any other treatment (control vs. inoculation). The sum of heat loss (i.e., area under the curve [AUC]) over the entire length of aerobic exposure (14 d) was impacted by substrate ($P<0.001$), as well as inoculation ($P<0.001$) and their interaction ($P<0.001$). CSF was most stable (smallest AUC), most likely due to the highest amounts of lactic and acetic acid, while inoculation had no impact. CCB and CFB lost more heat (larger AUC) but similar to CSF, inoculation had no effect on AUC. In contrast, inoculation of pure corn silage (Ccon vs. Cinoc) was very effective. It prolonged aerobic stability by about 4d and reduced the total heat loss of Cinoc by more than 50% compared to Ccon ($P<0.001$).

Conclusions

Mixed cropping of corn with common beans, field beans or sunflowers resulted only in moderate increases in CP. Inoculation with heterofermentative LAB led to prolonged aerobic stability in corn silage but the same inoculant was less effective in mixed corn silages. The findings underline, that silage inoculants have to be chosen substrate specific and mixed crop corn silages differ from pure corn silages in terms of chemical composition, fermentation characteristics, and aerobic stability.

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Characterization of *Fusarium* spp. Collected from Corn and Wheat in Wisconsin

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Keywords: *Fusarium*, deoxynivalenol, corn, wheat

Introduction

Fusarium spp. cause a number of devastating diseases that impact crops worldwide. In Wisconsin, members of the *Fusarium graminearum* species complex primarily cause ear and stalk rots in corn (*Zea mays*) and *Fusarium* head blight (FHB; scab) in wheat, resulting in significant yield losses (Wise et al., 2024; Alisaac & Mahlein, 2023). In addition, some *Fusarium* spp. produce trichothecenes which are toxic to both humans and animals (Awuchi et al., 2022). The specific trichothecene produced—3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), or nivalenol (NIV)—is called its chemotype (Pasquali & Migheli, 2014). Typically, *F. graminearum* is associated with ear rots of corn and FHB in wheat leading to DON accumulation in Wisconsin (Kovalsky et al., 2016). Considering that Wisconsin is a leader in dairy production, *Fusarium* and deoxynivalenol (DON) are important considerations for corn and wheat cropping systems in the state. This study aimed to identify *Fusarium* spp. associated with corn in Wisconsin and to compare them to previously reported *Fusarium* spp. populations in wheat.

Materials and Methods

In 2022 and 2023, 41 *Fusarium* spp. isolates were obtained from infected kernels of corn displaying ear rot symptoms (Chibuogwu, 2024). A region of the translation elongation factor (*EF-1α*) gene was amplified and sequenced to identify isolates to the species level (O'Donnell et al., 2009). Multiplex PCR primers for the trichothecene 15-O acetyltransferase (*TRI3*) and trichothecene efflux pump (*TRI12*) genes were used to identify the DON chemotype of each *F. graminearum* isolate (Ward et al., 2002). A total of 378 *Fusarium* spp. isolates were previously obtained and identified from FHB symptomatic wheat heads in 2016 and 2017 (Mueller et al., 2021). Species composition and chemotype of *Fusarium* originating from corn and wheat were compared. Finally, a phylogenetic tree was constructed using the *EF-1α* partial gene region of all 41 *Fusarium* spp. isolates from corn and a subset of 24 from wheat.

Results

The species breakdown of *Fusarium* spp. isolates from corn was 36.6% *F. proliferatum*, 29.3% *F. graminearum*, 9.8% *F. fujikuroi*, 9.8% *F. subglutinans*, 7.3% *F. verticilloides*, 4.9% *F. cerealis*, and 2.4% *F. sporotrichioides*. The 378 *Fusarium* spp. isolates collected from winter wheat fields were identified as 68.8% *F. graminearum*, 29.9% other, and 1.3% *F. culmorum*. Proportions of species originating from corn or wheat differed significantly ($X^2 = 289.06$, $df = 8$, $P < 0.01$). Additionally, there was a significant difference in the proportion of *F. graminearum* between corn and wheat ($P < 0.01$). There was no significant difference in proportion of *F. graminearum* chemotypes between corn and wheat ($P = 0.332$).

Conclusions

We found substantial evidence that *Fusarium* species community composition differs between corn and wheat in Wisconsin. Specifically, it appears that *F. graminearum* is more frequently associated with FHB than with corn ear rots. Although no difference was found in *F. graminearum* chemotypes between corn and wheat, populations should be continually evaluated to detect future shifts. Findings from this study will be used to inform management strategies for *Fusarium* in both corn and wheat and document current *Fusarium* species diversity in Wisconsin.

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Effect of Silage Additives Type and Dose on Alfalfa Silage Quality and Aerobic Stability

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Keywords: alfalfa silage, bacterial inoculant, chemical preservative, fermentation, aerobic stability

Introduction

Alfalfa is difficult to ensile due to its high buffering capacity and low water-soluble carbohydrate content (Sun et al., 2021). Ogunade et al. (2018) reported that poorly made or contaminated silages may contain pathogens that reduce animal performance. The aim of this study was to compare the effects of a biological inoculant (containing *Lactiplantibacillus plantarum*, *Pediococcus pentosaceus*, *Enterococcus faecium*) and a chemical preservative (containing formic acid, propionic acid, sodium formate, and sodium benzoate) at two different doses on fermentation quality and aerobic stability of alfalfa silage.

Materials and methods

Alfalfa chopped material (37.3% of dry matter) was ensiled in triplicates in special perforated bags with temperature monitoring. Inside each bag was a thermochron temperature sensor (iButton Device DS 1921G-FS) set to measure every 4 hours with an accuracy of 0.065 °C. The treatments were: control without additive, bacterial inoculant at doses of 1.5 and 3.0 g/t, chemical preservative at doses of 3 and 5 kg/t. After 33 weeks, fermentation parameters were analyzed and aerobic stability was tested.

Results

The bacterial inoculant significantly reduced pH (4.12 vs. 4.16; $P < 0.01$) and $\text{NH}_3\text{-N}$ content (27.6 vs. 28.1 mg N/100 g; $P < 0.01$) compared to control (Table 1). The chemical preservative slowed down the temperature increase during fermentation (peak 33.5-34.5°C after 7 weeks) compared to inoculant and control (38.7-39.7°C after 3-4 weeks). Higher doses of additives had no significant effect on fermentation quality. All variants showed good aerobic stability. Our results correspond with observations by Santos et al. (2023) regarding the effect of bacterial inoculant on pH and with findings by Davies et al. (2021) concerning temperature control using chemical preservatives.

Table 1: Fermentation quality parameters of silage variants

Parameter	Control	H1.5	H3.0	SF3	SF3b	SF5	SEM	P-value
pH	4.16 ^{ab}	4.12 ^a	4.12 ^a	4.18 ^b	4.18 ^b	4.18 ^b	0.01	<0.01
LA	3.06	3.07	3.06	2.99	3.05	3.03	0.03	NS
VFA	1.58	1.57	1.53	1.60	1.54	1.55	0.02	NS
LA/VFA	1.94	1.95	2.00	1.87	1.88	1.96	0.03	NS
$\text{NH}_3\text{-N}$	28.1 ^b	27.5 ^a	27.7 ^a	27.5 ^a	27.8 ^{ab}	27.8 ^{ab}	0.08	<0.01

LA = lactic acid in %; VFA = volatile fatty acids in %; $\text{NH}_3\text{-N}$ in mg N/100 g silage; H1.5, H3.0 = Harvest International pH at doses of 1.5 and 3.0 g/t; SF3, SF3b, SF5 = Silafor 2000 Plus at doses of 3 and 5 kg/t.

Means within a row (cultivar comparison) not followed by the same letter are different ($P < 0.01$).

Effect of microbial and chemical additives on the fermentation and aerobic stability of alfalfa silage ensiled at 2 dry matters was investigated by Li et al. (2021). Significantly better results were obtained when alfalfa was ensiled with 38 % of dry matter than with 46 % of dry matter. When comparing the results, it should be emphasized that the application of both inoculants (*Len. buchneri* 40788 and *Pediococcus pentocaseus* 12455) and the chemical preparation (sodium benzoate, potassium sorbate and sodium nitrite) increased the aerobic stability of alfalfa silage, but the additives used had a different composition.

Conclusions

The bacterial inoculant demonstrated better effects on fermentation parameters, while the chemical preservative more effectively controlled temperature during fermentation.

Properly preserved alfalfa silage showed good aerobic stability regardless of the type of additive used.

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Selection and Nutritive Value of Different Hybrids of Maize Silage

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Introduction

Numerous studies have evaluated the impact of advancing maturity, mechanical processing, application of additives on nutritive value and digestibility of maize silage (Andrae et al., 2001, Thomas et al., 2001, Jensen et al., 2005, Filya and Sucu, 2010, Hetta et al., 2012). Loučka and Jambor (2009) published differences in the content of selected nutrients and digestibility of whole plants in five hybrids with the same earliness (medium early FAO 300). Cherney et al. (2004) monitored fermentation characteristics of maize hybrids, selected to vary in dry matter (DM), crude protein (CP) and crude fibre digestibility (CFD), whereas Russell et al. (1992) demonstrated that the differences in the nutritional value of silage were not influenced only by individual hybrids, but also by methods of tillage and agro-technical measures.

The aim of our study was to compare the differences in the content of organic matter degradability (OMD) and neutral detergent fibre degradability (NDFD) for two maize hybrids grown for silage in farm conditions.

Material and Methods

At condition of agriculture cooperative AGROSPOL a.s., Knínice u Boskovic we planted two hybrids of maize (Ceplan and Cefin) for testing of nutritive value and quality. Maize hybrids produce firm CEZEA a.s. Čejč. Maize (planted on 10th May) was harvested at silage maturity on 25th September by the same harvester at the same day and pressed into a big plastic bag with the capacity of 1 tone of Fresh Matter (FM) for each plastic bag. On January next year we take away by hand sampler 5 samples from each plastic bag. Samples were taken according to the methods in Commission Regulation (EC) No 152/2009 (2009). Silage samples were analysed for fermentation quality (pH, lactic, acetic and butyric acid) using an IONOSEP 2001 analyser (RECMAN - laboratory systems, Ostrava, Czech Republic) according to Kvasnička (2000). Dry Matter (DM) was obtained from drying of silage at 60°C for 24 hours. Dried material was subsequently milled to pass through a one-millimetre sieve for laboratory analyses. NDF was assayed with a heat stable amylase and expressed inclusive of residual ash (aNDF) according to Mertens (2002). Dry Matter (DM), Ash, Crude Fibre (CF), and Crude Protein (CP) (N x 6.25) were determined as described by AOAC (2005). Organic Matter (OM) was calculated as DM minus Ash. Degradability of OM (OMD) and NDF (NDFD) was measured by *in sacco* method in the fistule rumen of dairy cows (McDonald, 1981). NEL act. was calculate by NDFD determinated by *in sacco* method and NEL tab. was calculate by NDFD 69,0 % which used agriculture laboratory according table numbers.

Results and Discussion

In the table 1 can see results of nutritive value of maize silage in plastic bags. The average values of DM, CP, CF and aNDF in both silages had a minimum difference.

Table 1 Nutrition value of maize silage, prepared from hybrid Ceplan

Sample	DM	CP	CF	aNDF	Starch	OMD	NDFD	NELact.	NEL tab.
1	28.4	8.9	22.0	44.3	27.5	71.7	50.7	6.32	6.79
2	28.9	8.7	21.2	43.9	27.6	73.5	54.6	6.45	6.80
3	29.8	8.7	21.4	44.8	27.2	75.0	56.4	6.49	6.81
4	28.9	9.0	20.1	43.7	26.7	73.2	51.3	6.40	6.81
5	30.4	8.6	21.1	44.0	31.1	74.0	55.3	6.47	6.81
Avg	29.3	8.8	21.2	44.1	28.0	73.4	53.6	6.43	6.80
SD	0.82	0.16	0.69	0.43	1.76	1.19	2.52	0.07	0.01

Table 2 Nutrition value of maize silage prepared from hybrid Cefin

Sample	DM	CP	CF	aNDF	Starch	OMD	NDFD	NELact.	NEL tab.
1	34.1	9.2	20	43.9	34.8	61.7	38.1	6.1	6.81
2	30.8	9.3	21.9	45.3	31.7	59.9	35.6	5.95	6.78
3	33.3	9.4	20.7	43.9	34.1	63.4	37.2	6.04	6.79
4	31.7	9.2	22.3	46.4	31.5	62.5	45.8	6.19	6.78
5	31.2	9.2	19.5	48.7	31.5	61.7	44.6	6.27	6.81
Avg	32.2	9.3	20.9	45.6	32.72	61.8	40.3	6.11	6.79
SD	1.43	0.1	1.20	2.01	1.60	1.30	4.62	0.13	0.02

Content of starch is significantly higher ($P < 0.05$) with hybrid Cefin (32.2 % DM) and lower with hybrid Ceplan (29.28 % DM). NDFD was significantly higher ($P < 0.05$) with hybrid Ceplan (53.65%) compared to hybrid Cefin (40.27%). Significantly higher OMD was with CeplanCeplan (6,43 NEL. Kg DM) than hybrid Cefin (6,11 NEL.kg DM).

This is reflected in the value of calculating the concentration of power in both silages netto energy of lactation (NEL), see Table 3. In calculating the energy value of maize silage in agricultural laboratories commonly used CFD of table value of 69%. If these tables digestibility of CF was used in both silages resulting concentration of power NEL had minimal differences. These minor differences are due to the fact that the content of organic nutrients in both silages was minimal. In our case, where we have established laboratory values of NDFD, the difference was 13.38%, and the resultant value was significantly ($P < 0.05$) higher 6.43 NEL MJ.kg⁻¹ DM. NEL of hybrid Ceplan, compared with hybrid Cefin, have had 6.11 MJ.kg⁻¹ DM.

Table 3 Nutritive value of maize silage made from hybrid CEPLAN and CEFIN

Hybrid	DM	CP	CF	aNDF	STARCH	OMD	NDFD	NEL	NEL tab.
CEPLAN	29.3	8.78	21.2	44.1	28.0 ^a	73.4 ^b	53.6 ^b	6.43 ^b	6.80
CEFIN	32.2	9.26	20.9	45.6	32.7 ^b	61.8 ^a	40.3 ^a	6.11 ^a	6.79

^{a,b} Means in the same column with different superscripts differ ($P < 0.05$).

Conclusion

The result of this experiment shows, that content of CP, CF, aNDF of maize silage of two silage hybrids is similar, but we determined that content of starch, OMD and NDFD was statistically different ($P < 0.05$) at this farm experiment. We can calculate, if the maize silage from hybrid CEPLAN had a higher energy content (6.43 MJ.kg⁻¹ DM) than CEFIN (6.11 MJ.kg⁻¹ DM). Cows which have intake of maize silage (10 kg of DM) can produce from this maize silage 20.6 kg resp. 19.6 kg of milk per day. For maize silage from CEPLAN hybrid it is 1 kg of milk more than from maize silage from hybrid CEFIN. According Oba and Allen (1999) 1% NDFD increase intake of 0.25 kg of DM. Increasing of intake of maize silage increase the energy intake and it means also higher production of milk.

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Effects of the Newly Selected Strain *Lentilactobacillus buchneri* DSM 34586 on Fermentation and Aerobic Stability Across Different Forages

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Keywords: aerobic stability, silage, inoculant, mini-silos, losses

Introduction

Lactic acid bacteria (LAB) inoculants are selected to enhance fermentation efficiency and to inhibit aerobic deterioration during both fermentation and feed-out phases (Muck *et al.*, 2018). However, the same inoculant can have varying effects on the fermentation profile depending on the type of ensiled materials, which may influence the aerobic stability of the silages. Among the LAB used to enhance the aerobic stability of silages, heterofermentative strains of *Lentilactobacillus buchneri* have been recognized as the most effective (Kleinschmit and Kung, 2006; Weiss *et al.*, 2016). In this study, we tested the capacity of the *Lentilactobacillus buchneri* DSM 34586 strain to alter the fermentation of whole-plant maize, grass, and alfalfa silages, aiming to evaluate its effects on aerobic stability.

Materials and methods

Silages were prepared from whole-plant maize with a dry matter (DM) content of 344 g kg⁻¹ and containing 89 g kg⁻¹ DM of water-soluble carbohydrates (WSC), from grass wilted to a DM content of 294 g kg⁻¹ and containing 51 g kg⁻¹ DM of WSC, and from alfalfa wilted to a DM content of 297 g kg⁻¹ and containing 29 g kg⁻¹ DM of WSC. The laboratory study on maize, grass, and alfalfa forages included two treatments: (1) Control (C), with no inoculant applied, and (2) *Lentilactobacillus buchneri* DSM 34586 treatment (LB), inoculated at rate of 100,000 CFU per gram of fresh maize, grass, or alfalfa. For each crop, ten 3-L mini silos per treatment were prepared. Silages were stored at room temperature (20.0 ± 1°C) for 90 days. After the designated storage period, the mini silos intended for fermentation analysis were opened, and fermentation quality was evaluated using gas-liquid chromatography. The silages were subsequently exposed to air by transferring them into open polystyrene boxes and storing in a controlled ambient condition for an aerobic challenge lasting 21, 23, and 27 days for maize, grass, and alfalfa silages, respectively. Aerobic stability was defined as the duration (in hours) for which the silage remained stable, i.e., before rising 3°C or more above ambient temperature. At the end of the aerobic stability test, pH and weight loss measurements were taken. Each mini silo served as the experimental unit for statistical analysis, with significance defined at $P \leq 0.05$. The data were analyzed with GLIMMIX procedure of the SAS Statistical Software using a Gaussian distribution for the response variables.

Results

The inoculant treatment did not influence the pH values of silage at opening (Table 1). However, after the aerobic exposure period, the pH was significantly lower ($P < 0.05$) in the silages treated with LB vs. C silages, indicating suppressed deterioration in the inoculated silages. Yeast and mold count at the end of fermentation period of the silages were significantly higher ($P < 0.05$) in the non-inoculated silages compared to those treated with *Lentilactobacillus buchneri* (DSM 34586), respectively. Similarly, lower ($P < 0.05$) numbers of yeasts and molds were found in the inoculated silages after the aerobic exposure

Table 1. Microbial contamination and pH value of the forages and silages untreated or treated with *Lentilactobacillus buchneri* (DSM 34586)

	----- Maize -----		----- Grass -----		----- Alfalfa -----	
	C	LB	C	LB	C	LB
	----- pH -----					
Harvest	5.91	5.91	6.70	6.70	6.50	6.50
Opening	3.84	3.85	4.36	4.36	4.88	4.83
Aerobic exposure	7.14	4.11*	9.12	4.81*	7.72	5.65*
	----- Yeast (Log ₁₀ CFU/g fresh matter) -----					
Harvest	5.60	5.60	5.70	5.70	5.60	5.60
Opening	2.16	1.12*	1.84	1.12*	1.97	1.52*
Aerobic exposure	7.60	4.22*	7.98	6.13*	7.40	6.84*
	----- Mold (Log ₁₀ CFU/g fresh matter) -----					
Harvest	5.30	5.30	5.40	5.40	5.20	5.20
Opening	2.11	1.12*	1.42	1.12*	1.86	1.18*
Aerobic exposure	8.89	4.87*	9.42	5.80*	8.19	6.56*

C–non-inoculated; LB–inoculated with *Lentilactobacillus buchneri* (DSM 34586); * – describe significant differences between treatments at $P < 0.05$.

The acetic acid content was significantly influenced by the application of LB (Table 2), showing an increase of 1.6 - 2.3 times ($P < 0.05$) in the inoculated silages. This also affected the lactic acid ratio and 1,2-propanediol concentrations, which were 54% to 2.3 times higher ($P < 0.05$) and 4 to 13 times lower ($P < 0.05$), respectively, in the non-inoculated silages. The LB treatment significantly reduced alcohol and ammonia-N concentrations ($P < 0.05$), as well as DM losses ($P < 0.05$). It also had a pronounced effect on delaying silage deterioration during aerobic exposure ($P < 0.05$).

Inoculated maize, grass, and alfalfa silages demonstrated 5.2 times, 3.5 times, and 1.2 time longer ($P < 0.05$) aerobic stability, respectively, and reached maximum temperatures that were 5.8, 7.6, and 4.0°C lower ($P < 0.05$) than those non-inoculated silages, respectively. Fresh weight loss during the aerobic exposure period was 2.6 times ($P < 0.05$), 93% ($P < 0.05$), and 8% lower for inoculated maize, grass, and alfalfa silages, respectively, compared to the non-inoculated silages.

Table 2. Fermentation and aerobic stability characteristics of the silages treated with *Lentilactobacillus buchneri* (DSM 34586)

Item	-----Maize-----		-----Grass-----		-----Alfalfa-----	
	C	LB	C	LB	C	LB
	----- Fermentation -----					
Lactic acid, g kg ⁻¹ DMc	38.74	33.76*	66.79	63.89	55.79	56.40
Acetic acid, g kg ⁻¹ DMc	20.84	32.76*	28.23	41.71*	24.72	56.67*
Lactic acid/Acetic acid ratio	1.86	1.04*	2.37	1.54*	2.26	1.00*
Alcohols, g kg ⁻¹ DMc	8.40	10.20*	8.15	8.49	10.96	9.16*
1,2-propanediol, g kg ⁻¹ DMc	0.24	3.15*	0.28	1.62*	0.39	2.52*
Ammonia-N, g kg ⁻¹ total N	64.64	50.02*	56.74	45.24*	69.72	55.88*
Dry matter loss, g kg ⁻¹ DMc	70.50	51.00*	81.00	67.00*	77.88	62.73*
	----- Aerobic stability -----					
Aerobic stability, hours	87.60	454.8*	129.6	450.0*	530.4	618.0
Maximum temperature, C°	30.6	24.8*	29.6	22.03*	27.4	23.4*
Fresh weight loss, %	10.3	4.0*	6.29	3.25*	5.45	5.05

C–non-inoculated; LB–inoculated with *Lentilactobacillus buchneri* (DSM 34586); * – describe significant differences between treatments at $P < 0.05$

Conclusions

The tested inoculant effectively shifted the fermentation process towards increased production of acetic acid and 1,2-propanediol, significantly enhancing the aerobic stability of maize and grass silage. Although alfalfa silage demonstrated high inherent resistance to aerobic spoilage, the application of *Lentilactobacillus buchneri* DSM 34586 did not further improve its aerobic stability. However, by inhibiting the growth of yeasts and fungi, the inoculant contributed to improved sanitary quality of the feed.

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Effect of Different Types of Silage on Milk Yield and Composition in Sri Lankan Dairy Cows

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Keywords: Silage, Maize Silage, Legume silage, Grass silage, Milk yield

Introduction

This study aims to evaluate the impact of different types of silage on milk yield and composition in dairy cows in Sri Lanka. Dairy farming is a significant sub sector of livestock in Sri Lanka, with over 300,000 farmers engaged in milk production. The objective was to assess the effect of silage on performance of dairy cows.

Materials and methods

Selection of Animals: Sixty dairy cows in mid to late lactation (140-305 days postpartum) were selected. The cows were homogeneous in terms of breed, age, and health status.

Experimental Design: The cows were randomly assigned to three groups of 20 cows each. Each group was fed a different type of silage:

Group 1: Maize silage

Group 2: Grass silage

Group 3: Legume silage

Feeding method: The cows were fed the respective silages ad libitum for a period of 12 weeks. All other dietary components remained constant across the groups.

Data Collection: Primary data were collected using a pre tested questionnaire

Milk Yield: Daily milk yield was recorded for each cow using a calibrated milk meter.

Milk Composition: Weekly milk samples were collected and analyzed for fat, protein, lactose, and other constituents using standard laboratory methods.

Statistical Analysis: Data were analyzed using ANOVA to determine the significance of differences between the groups.

Results

The results indicate that cows fed maize silage had the highest average daily milk yield, followed by those fed legume silage and grass silage. The higher energy content of maize silage likely contributed to the increased milk yield. Legume silage, with its higher protein content, resulted in higher milk protein levels compared to the other groups. Grass silage, while providing adequate nutrition, resulted in the lowest milk yield and composition metrics.

Table 1: Average daily Milk Yield by type of Silage fed to the dairy cows

Group	Average Daily milk Yiel (kg/cow)
Maize silage	18.3
Grass Silage Group	10.0
	15.7

Table 2: Milk Composition by type of Silage

Milk Composition	Maize Silage	Grass Silage	Legume Silage
Fat Content (%)	3.6	3.3	3.8
Protein Content (%)	3.2	3.1	3.4
Lactose Content (%)	4.7	4.5	4.8

Conclusions

The study concludes that maize silage is the most effective in enhancing milk yield, while legume silage improves milk protein content. Grass silage, although beneficial, resulted in lower milk production compared to the other silages. These findings highlight the importance of selecting the appropriate type of silage to optimize milk production and composition in dairy cows.

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Inoculant Improves Fermentation and Inhibits Clostridia in Difficult-To-ensile Forage with and Without Clostridia Challenge

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Keywords: grass-clover forage, homo- and heterofermentative bacteria, Clostridia, aerobic stability

Introduction

The aim of this study was to evaluate the effect of the unique homo- and heterofermentative inoculant commercialized as BioStabil Plus on fermentation quality and the prevention of Clostridia proliferation in difficult-to-ensile grass-clover forage.

The study was conducted both with and without a Clostridia challenge. Clostridia in silage can compromise feed value by breaking down protein and sugar, leading to the production of butyric acid and reduced silage quality. Clostridia can also negatively affect animal health and compromise the quality of food products. The experiment was conducted at Swedish University of Agricultural Sciences and followed the German DLG guidelines for the certification of silage additives (DLG 2017).

Materials and methods

First cut grass-clover herbage (73% grass on a dry matter basis) consisting of timothy (*Phleum pratense*), perennial ryegrass (*Lolium perenne*), meadow fescue (*Festuca pratensis*), red clover (*Trifolium pratense*), and white clover (*Trifolium repens*) was harvested with a mower-conditioner (at 8 cm height) at the beginning of June. Wilting was conducted in the laboratory by leaving the harvested material at room temperature until it reached 28% DM. The chop length was 30 mm. With 2.63% water-soluble carbohydrate (FM basis) and a fermentation coefficient of 28.7 [FC = $DM\% + 8 \times (WSC\% / \text{buffer capacity})$], the silage was classified as difficult to ensile.

Well-mixed samples were assigned to either an untreated control group or treated with the BioStabil Plus inoculant (*Lactiplantibacillus plantarum* DSM 19457, *Levilactobacillus brevis* DSM 23231, and *Lentilactobacillus buchneri* DSM 19455) applied at 2×10^5 cfu/g. The Clostridia challenge was conducted with *Clostridium tyrobutyricum* at 104 spores/g. The forage was ensiled in 1.75-L glass jars at a density of 153 kg DM/m³. The fermentation profile and aerobic stability were tested after 14, 21, 42, and 90 days. The Clostridia-challenged silage was tested on Day 90. Data were analyzed using Proc GLM and Wilcoxon Scores (Proc Npar1way, SAS).

Results

The addition of the BioStabil Plus silage inoculant significantly reduced the concentrations of ammonia-N, butyric acid, 2,3-butanediol, and ethanol, while significantly increasing the content of lactic acid, acetic acid, and 1,2-propanediol in the silage (Table 1). No proliferation of Clostridia was observed in silos treated with BioStabil Plus. BioStabil Plus significantly reduced silage pH by 33% compared to the control at the first opening (Day 14).

The silage inoculant also significantly decreased dry matter (DM) losses, from 48% at Day 14 to 54% at Day 90, compared to the control. In the Clostridia-challenged trial, there was a significant decrease in DM losses by 57% compared to the control. Silage treated with BioStabil remained stable from the first opening (Day 14) to the last opening (Day 90) during the 10-day aerobic stability test (Figure 1).

Conclusions

BioStabil Plus enhanced silage quality by promoting better fermentation, inhibiting Clostridia, preserving dry matter, and increasing aerobic stability in challenging ensiling conditions. These results highlight the dual benefits of improved nutritional quality for productivity and enhanced hygienic conditions for animal welfare.

Table 1. Fermentation profile of grass-clover silage (28% DM) with and without silage inoculant and without and with Clostridia challenge, opened at Day 90

Parame	Without Clostridia Chal				With Clostridia Challenge			
	ontrol	BSP	SEM	P-value	Control	BSP	SEM	P-value
DM loss	1.27 ^a	0.58 ^b	0.031	<0.001	2.37 ^a	1.01 ^b	0.017	<0.001
Ammonia-N*	1778 ^a	738 ^b	24.10	<0.001	1988 ^a	755 ^b	5.400	<0.001
pH	5.31 ^a	4.16 ^b	0.026	<0.001	5.48 ^a	4.19 ^b	0.016	<0.001
Lactic acid	1.74 ^a	4.17 ^b	0.055	<0.001	2.59 ^a	6.01 ^b	0.049	<0.001
Acetic acid	0.50 ^a	0.72 ^a	0.015	<0.001	0.98 ^a	1.10 ^b	0.013	<0.001
Propionic acid	0.66 ^a	0.06 ^a	0.011	<0.001	1.02 ^a	0.15 ^b	0.011	<0.001
Butyric acid	0.02	0.01	0.018	0.067	2.29 ^a	0.31 ^b	0.028	<0.001
Ethanol	0.74 ^a	0.42 ^b	0.010	<0.001	1.15 ^a	0.65 ^b	0.007	<0.001
2,3-butanediol	1.24 ^a	0.13 ^b	0.039	<0.001	2.29 ^a	0.31 ^b	0.028	<0.001
1,2-propanediol	0.10 ^a	0.16 ^{ab}	0.004	<0.001	0.10 ^a	0.16 ^b	0.003	<0.001
Clostridia spores					3.38	0		

^{a, b} for each experiment (without or with Clostridia), different superscripts indicate mean values within a row differ significantly (P<0.001)

* mg/L

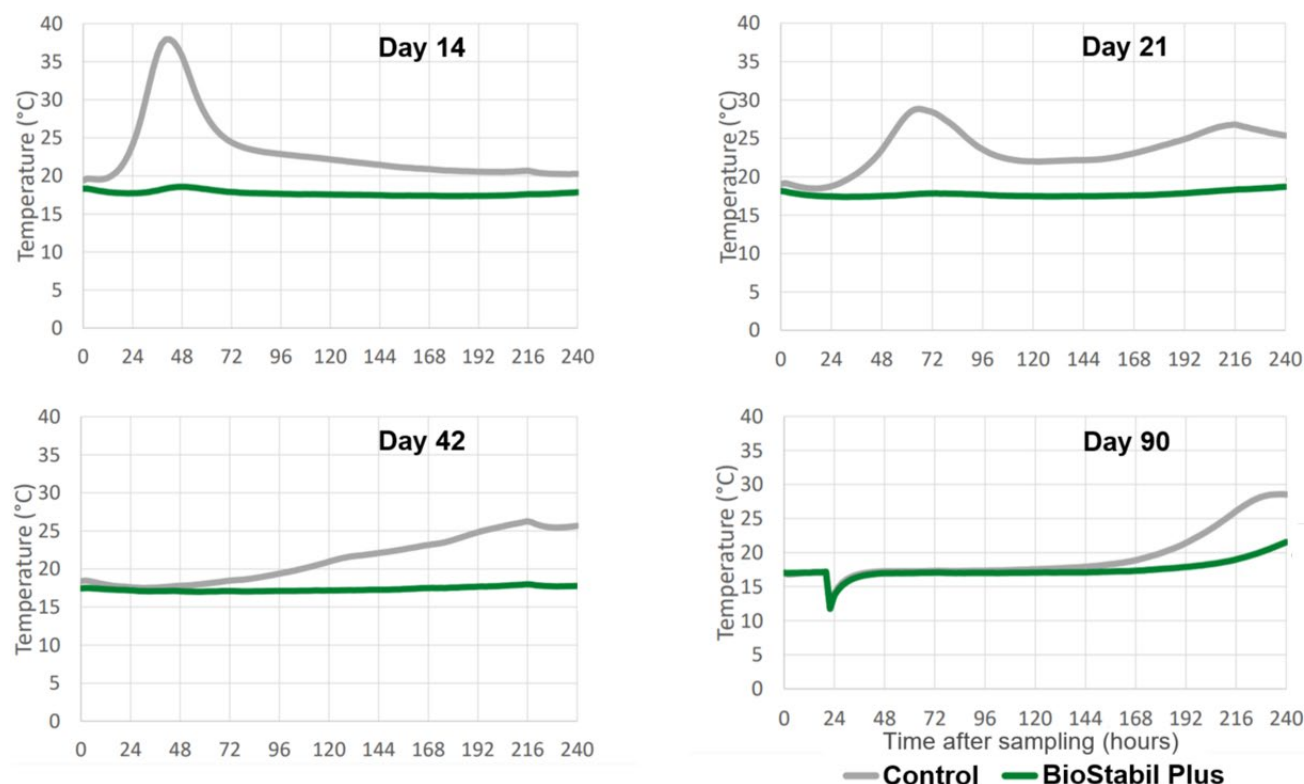


Figure 1. Longer aerobic stability in silage inoculated with BioStabil Plus at different opening days (Days 14, 21, 42 and 90)

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Pre-wilting Effects on Forage Soybean Silage

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Keywords: dry matter ratio, forage soybean, pre-wilting, silage pH, solar radiation

Introduction

In Japan, imported feed such as alfalfa (*Medicago sativa* L.) hay and soybean meal have been used as a high-protein feed. The rising price of imported feed has led to a growing demand for a stable supply of domestically produced high-protein feed. To develop technology to ensure a stable supply of domestically produced high-protein feed, the authors considered it useful to utilize soybean (*Glycine max* (L.) Merr.), which is widely cultivated as feed worldwide (Asekova et al., 2014). In Japan, it should be harvested as whole crop silage (WCS) because rainy weather makes it difficult to prepare as hay. Studies assessing the cultivation of soybean WCS have been conducted in Japan (Kaneko et al., 2011). In this cultivation system, Italian ryegrass aftermath is used as cover crop for forage soybean because there is no registered herbicide for forage soybean production in Japan. Additionally, investigations of the feeding of soybean WCS have been conducted in Japan (Touno et al., 2014). Nevertheless, the effects of harvest management, especially field wilting on silage quality, remain unclear. From this study, we clarified effects of pre-wilting time on the dry matter ratio and silage fermentation of forage soybean under the assumption that the harvested foliage would be wrapped in a roll bale and plastic-wrapped on the day of cutting.

Materials and Methods

This study was conducted at NARO Kyushu Okinawa Agricultural Research Center, Kumamoto, Japan (32°53'N, 130°44'E, 78 m a.s.l.) in 2024. The soil, which was heavy clay (Hydric Pachic Melanudands by Soil Taxonomy, 1999), was categorized as Andosol by the Soil Classification System of Japan (The Fifth Committee for Soil Classification and Nomenclature, 2017). Seeds of the 'Akioba 3' cultivar of Italian ryegrass were broadcast to fields (20 m × 65 m) on February 28, 2024, followed by tillage with a plow and rotary cultivator. Then the soil was compressed using a roller. The first crop of Italian ryegrass was harvested on 9 May. Soybean seeds were sown directly into the Italian ryegrass sod without cultivation, with 48 rows to a plot at 30 cm row spacing, using a commercial no-till planter (NTP-2AF; Agritechno Search Co., Ltd., Himeji, Hyogo, Japan) on 17 May.

During the three sunny days of September 2–4, soybeans were cut with a mower at 1 pm every day (8 rows per day). Then samples were taken immediately after cutting, and 1 hr, 2 hr, and 3 hr later. The collected samples were shaken in the laboratory and were separated into soybean foliage, shed soybean leaves (that fell off when cut by the mower and could not be picked up by the roll baler), and others (include weed and dead materials). The fresh weight of each was measured at three rows with 30 cm width. After they were then dried at 70°C for more than 72 hr, the dry weight was measured to ascertain the dry matter ratio. Only for soybean foliage, after measuring the fresh weight, a portion was separated for laboratory-scale silage testing using vacuum packs. The remainder was dried.

For laboratory-scale silage tests, soybean foliage samples were shredded to a 47 mm cutting length using a cutting machine (CX-201; Yamamoto Co., Ltd., Tendo, Yamagata, Japan). Then 200 g of shredded soybean foliage was placed in a vacuum bag (24 cm × 350 cm, 75 µm thickness, Hiryu N-10; SB PAX Co., Ltd., Ageo, Saitama, Japan). Eight bags for each sample were sealed using a vacuum machine (SQ-203S; Asahikasei Pax Co. Ltd., Tokyo, Japan). The bags were stored in a storage room without temperature control for 60 or 90 days (4 bags for each sample). After being sealed 60 or 90 days, the bags were opened, and silage soybean foliage was used to evaluate pH measurement. The obtained bag contents were extracted with 100 ml of reverse distilled water per 25 g of contents overnight at 4°C and were then filtered with a 5A quantitative filter paper (Advantec Toyo Kaisha, Ltd., Tokyo, Japan). The pH of the extracts was measured using a pH meter (PH71; Yokogawa Electric Corp., Musashino, Tokyo, Japan).

Dry matter ratio data were analyzed using R (ver. 4.2.0) with plant parts and time after cutting as fixed effects. Silage pH data were analyzed using R (ver. 4.2.0) with storage periods and time after cutting as fixed effects. Two-way analysis of variance (ANOVA) was used for testing. When the effect of time after cutting was inferred as significantly different ($P < 0.05$), Tukey's multiple comparison test was applied.

Results

All three days of this study were sunny. The average temperature was approximately 31°C for each of the three hours. The values of solar radiation and relative humidity decreased gradually as time progressed (Table 1).

Table 1. Weather conditions of the pre-wilting periods

Weather conditions	Time after cutting		
	0–1 hour	1–2 hours	2–3 hours
Temperature (°C)	31.8	31.9	31.6
Relative humidity (%)	60.1	58.8	57.0
Solar radiation (MJ/m ²)	2.9	2.6	1.9
Duration of sunshine (hr)	1.0	1.0	1.0

The soybean growth stage at the time of cutting was from R6 (full seed) to R7 (beginning maturity). The dry matter ratio of both soybean foliage and shed leaves were about 28.5% at the time of cutting (0 hr), increasing to about 35% within 3 hr ($P<0.01$). Significant difference was found between soybean and shed leaves ($P<0.05$). At 1, 2, and 3 hr after cutting, soybean foliage showed a lower value than shed leaves (Table 2).

Table 2. Dry matter ratio of soybean foliage and shed soybean leaves with few hours pre-wilting

Plant parts		Time after cutting			
		0 hour	1 hour	2 hours	3 hours
Soybean foliage		28.7c	30.9b	32.5ab	34.4a
Shed soybean leaves		28.4b	32.6ab	35.0a	36.2a
P value	Soybean parts	$P<0.05$			
	Time after cutting	$P<0.01$			
	Interaction	$P=0.46$			

† Means within a line (time comparison) not followed by the same letter are different ($P<0.05$).

From the silage test using degassing bags, the pH was found to be 5.1–5.4. No difference was found related to the storage period. Longer time after cutting was associated with lower pH values, but the difference was not significant (Table 3).

Table 3. pH of silage soybean foliage with few hours pre-wilting after storage

Storage periods		Time after cutting			
		0 hour	1 hour	2 hours	3 hours
60 days		5.4	5.3	5.2	5.1
90 days		5.4	5.4	5.3	5.2
P value	Storage periods	$P=0.58$			
	Time after cutting	$P=0.11$			
	Interaction	$P=0.96$			

Conclusions

After drying for three hours on a sunny autumn afternoon, the silage was dried to a dry matter ratio of approximately 35%. Drier silage was found to be associated with lower pH, suggesting that higher quality silage can be produced. Other assessments of silage quality are also needed.

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Comparison of Effects of Silage Additives on the Fermentation Quality and Dynamics of Alfalfa Silage

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Keywords: alfalfa, domestic microbial inoculants, fermentation dynamics

Introduction

Hay production is highly weather-dependent, which presents significant challenges. Furthermore, the nutritional value of alfalfa hay can deteriorate during transportation and storage. Ensiling, however, can improve nutritional content and palatability through anaerobic fermentation of carbohydrates by lactic acid bacteria (LAB), where the pH is lowered to suppress undesired microorganisms such as *Escherichia coli*, yeast, and mold [1]. Ensiling has become a common and effective method for the long-term preservation of forage for livestock. Alfalfa (*Medicago sativa*) is rich in protein, had excellent fiber content, and offer high yield, making it a high-quality forage for ruminants. It is considered one of the most important forages worldwide [2]. However, the production of alfalfa silage faces significant challenges due to the low number of epiphytic LAB, low WSC content, and strong buffering capacity [3]. Consequently, additives are often necessary to improve the quality of alfalfa silage. Thus, the objective of this study was to compare the fermentation quality and dynamics in alfalfa silage treated with commonly used additives, including *Trichoderma* cellulase enzyme, the safety chemical additives sodium diacetate (SDA) (6 g/kg fresh matter), and popular microbial inoculants (TS, SK, KL, BSM, and LP).

Materials and methods



Results

The pH decreased during the ensiling, with a significantly faster rate of decline observed in the first 7 days. After 14 days, the pH tended to stabilize. On the 1st day of ensilage, TS and SDA showed a more rapid decline, while the microbial inoculant-treated silage had a lower pH starting from the 3rd day. After 60 days, the highest pH was recorded in the control group, followed by BSM. WSC followed a trend similar to pH, decreasing as the fermentation period progressed. On the 1st day, WSC content was highest in the SDA-treated silage, and no lactic acid (LA) was detected, suggesting that the rapid pH decrease could be attributed to the release of acetic acid (AA) molecules from SDA. Starting from the 3rd day, no significant differences in WSC content were observed in silages treated with microbial inoculants, while the control group consistently had the highest WSC content, except on the 60th day. LA and AA exhibited contrasting trends in relation to pH. The LA concentration in TS-treated silage was the highest on the 1st day, explaining the lowest pH and WSC content observed. From 3 to 14, no significant differences were founded in LA content among the microbial inoculant-treated groups. The control group consistently had the lowest LA content throughout the ensiling period. Only SDA exhibited AA on the first day, which coincided with the low pH value. In the first 7 days, AA content in the SDA group was significantly higher than in other treatments. However, from day 14 onwards, the AA content in the control group became the highest, followed by SDA and CELL. Propionic acid was detected only in the control group and not in any other treatments. The concentration of ammonia nitrogen (NH_3/TN) increased with ensiling duration and stabilized on the 30th day. A rapid increase in NH_3/TN was noted during the first 7 days. The control group had the highest NH_3/TN concentration, while KL most significantly inhibited NH_3/TN production, followed by SDA and LP.

Conclusions

In summary, SDA demonstrated the highest potential for improving the fermentation quality and dynamics of alfalfa silage, while KL stood out as the most effective among the five commercial microbial inoculants, outperforming cellulase.

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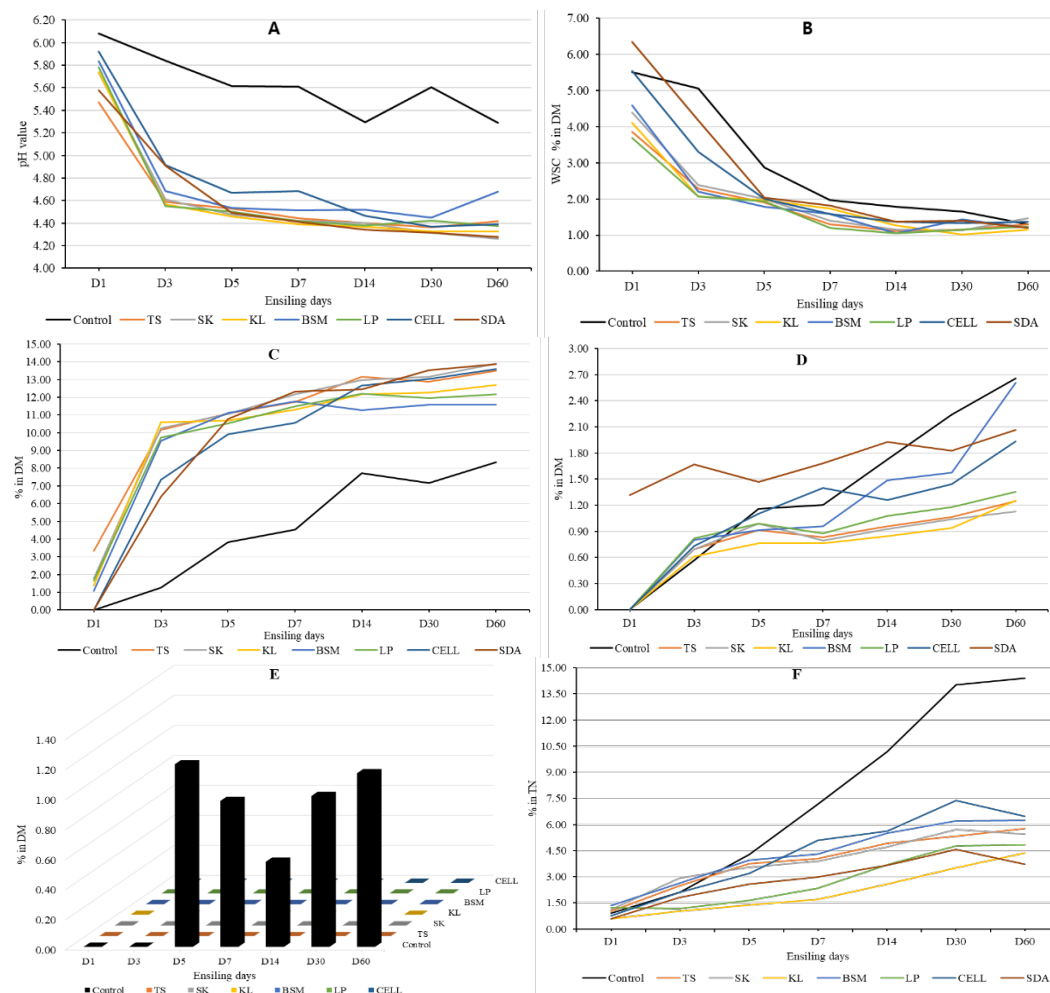


Figure 1. Fermentation quality and dynamics of alfalfa silage after 60 days of ensiling. pH value (A), Water-soluble carbohydrates (B), Lactic acid (C), Acetic acid (D), Propionic acid (E), Ammonia nitrogen/ total nitrogen (F).

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Dynamic Effects of Applied Homofermentative Lactobacilli on Wet Grass Silage with Regard to Fermentation Quality and Mass Losses

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Keywords: fermentation losses, butyric acid, ammonia-N, silage quality

Introduction

Crops ensiled wet are prone to effluent losses and clostridial fermentation, both reducing the feed value of the silage (Muck et al., 2003). Therefore, ensiling wet grass silage is a practical challenge to preserve the nutrients quantitatively and qualitatively for a longer period by inactivating microbial and feed-specific enzymes, i.e. to limit the decomposition processes to an unavoidable or desirable extent (Steinhöfel et al., 2023). At Austrian dairy farms, the average amount of butyric acid (BA) of first cut grass silages has been above 12 g/kg DM (SD 10 g/kg DM) in the past ten years, due to low nitrate and too high clostridia-level in fresh grass. At HBLFA Raumberg-Gumpenstein (E: 14°05'54,63", N: 47°29'44,40"), an ensiling study with very slightly wilted grass (DM content 238 g/kg) was carried out in 2024 on two variants - V1: control without additives and V2: A mixture of new selected homofermentative lactic acid bacteria (LABho) with focus on wet grass silage in order to proof the effect on fermentation quality and mass losses under challenging conditions.

Material and Methods

Forage data: Permanent grassland of first growth, >95% grasses, harvest date 10-05-2024, particle length 5-10 cm (cut by harvest wagon), forage temperature in the unloaded pile above 30°C. A quantity of 0.1 to 0.2 kg DM was ensiled in three replications in vacuum-bags (VB: 295 × 365 cm) to observe fermentation processes and losses after 7 and 30 days of storage. Plastic wide necked barrels (WNB 60 litre) were filled with 10 × 4 kg forage, in sum 6.8 to 7.5 kg DM material. WNB silos were weighed, opened and sampled after 87 and 192 days of storage. The silage additive V2 (LABho-mixture of *L. plantarum*, *L. lactis*, *L. paracasei* and *L. huelsenbergensis*) was applied with a spray bottle (target 250,000 CFU/g FM). VB and WNB were stored at ambient temperature of 10 to 18°C. Determination of DM, nutrients, pH-value and volatile organic compounds (VOC) was performed with wet chemistry methods (VDLUFA, 2012). DM and nutrient correction of the silages was carried out by calculations of Weißbach & Kuhla (1995). According to Köhler et al. (2019), the gravimetric difference method total-in vs. total-out was used to evaluate losses of fermentation. The protein loss in the silage was determined by subtracting of the mass of NH₃-N from total N, as NH₃-N is formed during N-degradation. Lactic acid bacteria were enumerated in accordance with the EN 15787 (2022) standard.

Results and Discussion

PH was reduced significantly after 7, 30, 87 and 192 days when using the LABho-mixture V2 (Table 1). WNB samples of V1 exhibited extreme butyric acid fermentation (48 resp. 53 g BA/kg DM) and thus a very poor silage quality. V2 significantly improved the conservation success (18 g BA/kg DM). Despite a very good pH-value reduction in V2, BA in WNB was above the DLG orientation value of < 3 g BA/kg DM (DLG, 2025), probably because of warmed ensiling material. Possibly, BA formation was below 1 g/kg DM in VB samples, because forage cooled down faster in VB than in WNB and prevented clostridia growth. V2 significantly reduces acetic acid and ethanol contents (Table 1), indicating the proliferation of the homofermentative LAB. NH₃-N formation increased in V1 after 192 days of storage up to 19% of total N, while treatment with V2 significantly decreased the NH₃-N content to 10.5%, but the orientation value (DLG 2025: < 8% NH₃-N) was still exceeded by up to 3%. However, the most notable success of V2 was the significant reduction in DM, protein and energy losses by more than half compared to V1. The level of DM losses in V1 (11.7% resp. 13.1%) was above the average of wet silages, but that of V2 (4.9% resp. 3.7%) can be compared with favourable conditions of wilted silages (Resch et al., 2025). V2 was able to limit the protein loss to 10-12%, while the loss during storage in V1 increased up to 25% (Table 1). Before the ensiling process starts, log₁₀ 3.57 CFU/g FM natural LAB were detected on the forage. After 87 days of storage, both variants showed comparable values (V1: log₁₀ 8.45, V2: log₁₀ 8.58 CFU/g FM), while the number of LAB in V2 decreased to log₁₀ 7.57 CFU/g FM compared to V1 (log₁₀ 8.15 CFU/g FM) after 192 days. This means that the ensiling process was accelerated in V2, and the LAB reached the stationary phase earlier.

Table 9: Impact of treatment and storage duration on ingredients, losses and fermentation quality of slightly wilted grass silage (treatments: V1 = control without additives; V2 = added LABho-mixture)

parameter	abb	unit	forage	silage 7 d (VB)		silage 30 d (VB)		silage 87 d (WNB)		silage 192 d (WNB)	
				V1	V2	V1	V2	V1	V2	V1	V2
dry matter	DM	g/kg FM	237,7	235	238	223	231	216 ^a	229 ^b	213 ^a	232 ^b
ammonia	NH ₃	% of N	0	9.6 ^b	7.9 ^a	15.0 ^b	9.3 ^a	18.3 ^b	11.0 ^a	19.4 ^b	10.5 ^a
metabolic energy	ME	MJ/kg DM	10,5	-	-	-	-	10.5 ^a	10.6 ^b	10.6 ^a	10.8 ^b
DM loss	D-L	%	0	0.9 ^b	0.1 ^a	7.3 ^b	3.3 ^a	11.7 ^b	4.9 ^a	13.1 ^b	3.7 ^a
protein loss	P-L	%	0	8.8 ^b	6.9 ^a	17.4 ^b	9.5 ^a	25.7 ^b	11.7 ^a	24.5 ^b	10.3 ^a
ME loss	M-L	%	0	-	-	-	-	12.8 ^b	4.1 ^a	12.4 ^b	1.3 ^a
pH value	PH		5.73	5.10 ^b	4.90 ^a	4.89 ^b	4.17 ^a	4.82 ^b	3.92 ^a	4.94 ^b	3.98 ^a
lactic acid	LA	g/kg DM		100 ^b	95.8 ^a	53.9 ^a	91.7 ^b	38.0 ^a	171 ^b	28.0 ^a	137 ^b
acetic acid	AA	g/kg DM		13.1 ^b	5.5 ^a	11.7 ^b	6.8 ^a	13.7 ^b	7.7 ^a	13.2 ^b	5.0 ^a
butyric acid	BA	g/kg DM		2.5 ^b	0.3 ^a	18.9 ^b	0.9 ^a	47.8 ^b	17.9 ^a	53.5 ^b	17.9 ^a
ethanol	ETH	g/kg DM		9.6 ^b	6.4 ^a	17.0 ^b	7.1 ^a	23.1 ^b	10.4 ^a	28.1 ^b	14.1 ^a

Conclusions

In the present study, the application of LABho led to significantly reduced losses of DM, protein and energy under challenging conditions of wet grass silage (DM 238 g/kg). In addition, the formation of butyric acid could only be reduced by V2, but not completely prevented in the WNB. Further precise studies are required to confirm the positive effects of the used biological silage additive based on new selected LABho for difficult to ensile forage, such as wet grass silage.

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Effect of Different Silage Inoculants on Fermentation and Protein Quality in Grass Silage

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Keywords: protein quality, silage inoculants, nitrogen efficiency

Introduction

Germany has committed to reducing ammonia emissions by 29% in 2030 compared to 2005 levels (EU, 2006). Approximately 92% of these ammonia emissions come from agriculture (Fuß et al., 2023). Improving the efficiency of nitrogen use is important to enable sustainable dairy farming in the future (Dinn et al., 1998). Analyses at the level of dairy farm production processes have shown that there is a large potential for emission savings in feed production. One of the main reasons for the low level of nitrogen utilization in feed production is the extensive protein degradation during ensiling, which occurs via two pathways (McDonald et al., 1991; Zehetmeier et al., 2017). In the first phase (proteolysis), plant enzymes degrade protein to non-protein nitrogen. The second phase (desmolysis) is mainly carried out by enterobacteria and clostridia, where amino acids are deaminated to ammonia and organic acids or decarboxylated to CO₂ and amines (Ohshima and McDonald, 1978). Other authors have reported a reduction in protein degradation with the use of bacterial silage additives (Winters et al., 2001). Therefore, the aim of this study was to evaluate whether silage inoculants based on homofermentative and heterofermentative lactic acid bacteria (LAB) improve the fermentation and protein quality of grass silage.

Materials and methods

Third cut grass material (Tall fescue; dry matter (DM): 30.8 %; water soluble carbohydrates (WSC): 13.4% DM; crude protein (CP): 13.7 % DM) was ensiled in laboratory mini silos (1.5 L). Three treatments were carried out in three replications:

CON: Untreated control

INO1: A mixture of homo - and heterofermentative LAB (*Bonsilage Speed G*: *L. buchneri*, *L. diolivorans*, *L. plantarum*; application rate: 250,000 CFU/ g FM)

INO2: A mixture of homo - and heterofermentative LAB (*Bonsilage Fit G*: *L. buchneri*, *L. plantarum*, *L. rhamnosus*; application rate: 300,000 CFU/ g FM)

The glasses were opened after 1, 3, 5, 7, 14 and 90 days of storage at 20°C. Measured parameters: pH value and NH₃-N (via NH₃ electrode) at day 1, 3, 5 and 7; DM, CP and fermentation pattern (via HPLC) at day 14 and 90. For statistical evaluation the medians were calculated and the non-parametric Mann Whitney U-Test was performed on the parameters to test for significant differences ($P < 0.05$) between the control and the treated silages.

Results

The pH values of the treated silages (INO1 and INO2) were significantly lower than those of CON at all opening days (OD) - see figure 1. Similarly, the NH₃-N contents in % fresh matter of INO1 and INO2 treated silages were significantly lower than those of the control at all ODs (INO1/INO2/CON – OD1: 0.01/0.01/0.03; OD3: 0.01/0.01/0.05; OD5: 0.02/0.02/0.06; OD7: 0.03/0.04/0.07; OD14: 0.05/0.05/0.08; OD90: 0.07/0.07/0.10). This significant difference was also confirmed for NH₃-N of total N at OD14 and OD 90 (INO1: 6.67% and 9.47%; INO2: 6.43% and 9.54%) compared to CON (10.9% and 14.1%). Lactic acid, acetic acid and 1,2-propanediol were significantly higher for INO1 and INO2 compared to control at OD14 and OD90. Differences were also found for butyric acid at OD90, with the control (0.74% DM) showing significantly higher values than INO1 (0.03% DM) or INO2 (0.00% DM). The medians of the results of the silage parameters are presented in table 1. It is obvious that the extent of protein degradation during ensiling process was affected by both LAB treatments INO1 and INO2. It is assumed that the faster and deeper pH reduction reduced the activity of plant proteases and suppressed undesirable protein- degrading bacteria, which was also found by Slottner and Bertilsson (2006) and McDonald et al. (1991).

Figure 4: pH value of the control (CON) and the LAB-mixture (INO1 and INO2) silages at all opening days (OD).

*: indicates significant difference between CON and INO1/INO2 ($P < 0.05$).

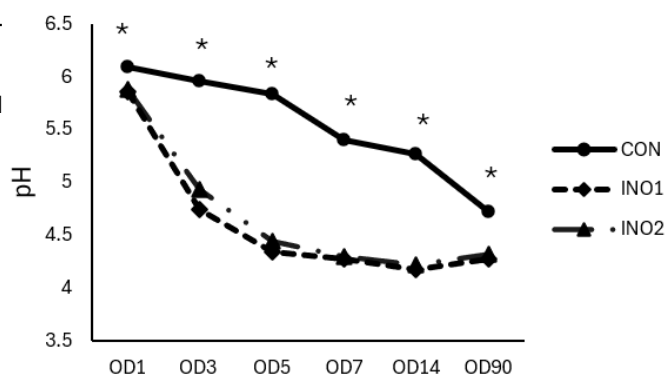


Table 1. Medians of the silage parameters of the grass silage at opening day (OD) 14 and 90 for control (CON), and LAB-mixture treatments INO1 and INO2.

Parameter		OD14			OD90		
		CON	INO1	INO2	CON	INO1	INO2
DM _c	%	30.4	30.5	30.9	29.7	30.1	29.9
CP	% DM	15.0	15.2	15.6	14.9	15.1	15.1
NH ₃ -N	% Total-N	10.9	6.67*	6.43*	14.1	9.47*	9.54*
LA	% DM	0.86	3.53*	2.97*	2.36	4.28*	4.02*
AA	% DM	0.26	1.58*	1.03*	1.75	2.65*	2.81*
BA	% DM	0.00	0.00	0.00	0.74	0.03*	0.00*
1,2PD	% DM	0.00	0.23*	1.33*	0.10	0.50*	2.31*

DM_c = DM corrected; CP = Crude protein; NH₃-N = Ammonia nitrogen; LA = Lactic acid; AA = Acetic acid; BA = Butyric acid; 1, 2-Propandiol; *: symbolize significant differences to control (P<0.05)

1, 2-

Conclusion

The present study demonstrates the beneficial effects of two LAB-mixtures containing homofermentative and heterofermentative bacteria on fermentation and protein quality of grass silage. Thus, LAB-mixtures should be used to minimize protein degradation in grass silage, increase N efficiency at farm level, and therefore have the potential to be part of an ammonia reduction strategy in dairy farming.

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Influence of *L. diolivorans* Inoculant on the Carbon Footprint of Corn Silage in a Biogas Plant

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Keywords: carbon footprint, silage inoculant, biogas, corn silage

Introduction

The use of silage additives in corn silage has already been investigated, showing that the additive contributes to enhancing fermentation efficiency, inhibiting the proliferation of yeasts, reducing spoilage, and stabilizing the silage (Herrmann et al. 2011). However, there is limited research about how silage additives influence the carbon footprint of corn silage used for biogas production. This study investigates the impact of a silage inoculant based on homo- and heterofermentative lactic acid bacteria (LAB) containing *L. diolivorans* on the carbon footprint of corn silage in a biogas system.

Materials and methods

The corn (36.2 % DM) used in this study was harvested on a dairy farm in north-western Germany in September 2023. The fresh material was weighed and stored into two silos: control ("untreated silage"), and test ("treated silage") - inoculated with a mixture of homo- and heterofermentative LAB (*L. diolivorans*, *L. buchneri*, and *L. rhamnosus*: application rate of 250.000 CFU/ g FM). Silos were opened after 16 weeks and used in dairy cows' rations. Fresh matter losses during anaerobic ensiling process and aerobic phase during feed-out were calculated as weight difference from harvested and fed-out amounts. Silage treatments were monitored weekly for three months to determine: pH, lactic and acetic acid concentration, number of yeasts and moulds, aerobic stability according to Honig (1990), and biogas production, determined by laboratory-scale batch tests according to VDI 4630. Statistical analyses were performed using OriginPro, and results are reported as mean values $\pm$ standard deviations (SD). Comparisons between the two groups were conducted with significance set at $p < 0.05$. The carbon footprint quantified greenhouse gas emissions associated for treated and untreated silage using a life inventory (LCI) approach, following ISO 14044 standards. Data included weight losses, laboratory results (corn silage, and liquid fermentation substrate), batch test outputs, agricultural inputs (fertilizer, energy), and biogas plant operational data. The LCI was developed using Open LCA, ecoinvent and the free database, *bioenergiedat*, containing information on bioenergy supply chains in the German context. Data regarding emissions were collected from IPCC (2019). The selected impact assessment methodology was IPCC 2021, with the chosen impact category being Global Warming Potential (GWP 100 non-fossil CO₂ uptake and emission). This category accounts for both fossil carbon flows and biogenic carbon.

Results

The treated silage showed significantly improved quality compared to the untreated silage (see Table 1).

Table 1. Characterization of the treated and untreated silage used in this study.

Parameter	Unit	Untreated silage	Treated silage
Weight losses	% FM	19.75	7.59
DM	g / kg	331.77 $\pm$ 26.9 ^A	348.01 $\pm$ 22.09 ^A
pH	-	3.66 $\pm$ 0.11 ^A	3.83 $\pm$ 0.08 ^B
Lactic acid	% DM	5.79 $\pm$ 1.97 ^A	4.31 $\pm$ 1.01 ^B
Acetic acid	% DM	1.40 $\pm$ 0.33 ^A	3.58 $\pm$ 0.48 ^B
Yeasts	CFU/ g FM	27200 $\pm$ 18074 ^A	180 $\pm$ 130 ^B
Moulds	CFU/ g FM	192060 $\pm$ 305436 ^A	100 $\pm$ 0 ^A
ASTA	h	72.40 $\pm$ 16.99 ^A	223.20 $\pm$ 16.35 ^B

DM= Dry Matter, FM = Fresh matter, ASTA = Aerobic stability time assessment, CFU = Colony forming unit

^{A,B}The letters show the significant difference between treated and untreated silage for each parameter; different letters indicate significant differences for $p < 0.05$ according to the Tukey Test.

The key findings include a 62% reduction in weight losses for treated silage compared to untreated silage. Additionally, an extended aerobic stability (ASTA) and a reduced number of yeasts and moulds were observed for treated silage versus untreated silage. In addition, biogas batch tests revealed a 3% higher methane yield for LAB treated silage (367.14 $\pm$ 19.81 NI/kg_{TS}) compared to untreated silage (355.89 $\pm$ 18.70 NI/kg_{TS}). Total biogas production also increased by 2% reaching (680.21 $\pm$ 30.38 NI/kg_{TS} vs 664.91 $\pm$ 27.97 NI/kg_{TS}). Calculated based on 1 kg DM of ensiled corn and accounting the observed losses, the treatment produced 22,5% more biogas overall. The carbon footprint results, presented in Figure 1, indicate that emissions from the LAB treated silage during the ensiling process are approximately 14.5% lower than those from untreated silage, primarily due to reduced emissions associated with weight losses. For biogas production, the carbon footprint of LAB treated silage was 18.2% lower compared to untreated silage, considering the weight losses during ensiling. The ensiling process accounts for all steps from corn cultivation to delivery at the biogas plant, while the biogas production process includes corn cultivation, ensiling, and generation of heat and electricity from the biogas. These results highlight the significant impact of ensiling losses on the overall environmental footprint and emphasize the potential of LAB treated silage to

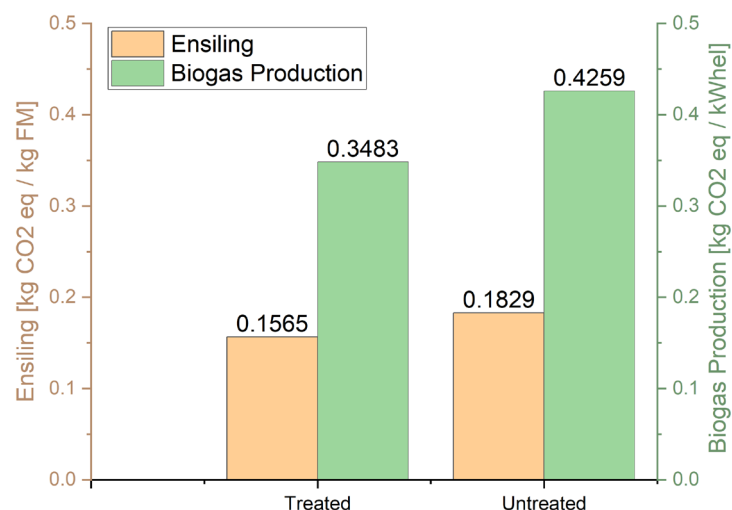


Figure 1. Greenhouse gas emissions on a CO₂-equivalent basis for ensiling process of corn silage (treated vs. untreated) and biogas production fed with corn silage (treated vs. untreated).

Conclusions

The use of a LAB mixture containing *L. diolivorans* improved silage quality significantly, with a reduction in weight loss, enhanced dry matter retention, and greater aerobic stability. These improvements translated into higher methane yields for biogas production, especially when weight losses were taken into account. Thus it was shown that the use of a LAB mixture containing *L. diolivorans* has the potential to reduce carbon footprint of biogas production in plants fed with corn silage.

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Recent Advances in and Future Recommendations for Silage Inoculant Technology

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Keywords: silage, inoculant

Summary

This presentation will briefly discuss the early history, recent findings, and potential future directions of improving silage fermentation with select bacterial inoculants.

Discussion

Select beneficial bacteria have been used as inoculants to improve the quality of silage fermentations for decades. Early efforts focused on adding classical homolactic acid bacteria that produced faster fermentations which decreased pH rapidly, minimizing the growth of undesirable microorganisms and their end products. The result was simplistically more efficient fermentations with higher recoveries of nutrients, energy, and dry matter. Research followed on adding exogenous enzymes (not bacterial inoculants) that could improve nutrient digestion of silage in the rumen. The seminal research of Nsereko et al. (2008) was significant as it showed that addition of bacteria added to forage at ensiling, produced ferulic acid esterase that improved ruminal fiber digestibility of silages. However, controlling the effectiveness of this organism to consistently and effectively improve fiber digestion requires more research. In addition, we need to understand the optimum amount and type of fibrolytic enzymes required as to not overly pre-digest fiber before it enters the rumen, which may result in excessive free sugars, aerobic stability issues, and the possibility of high levels of effluent.

The early approach to improving the aerobic stability of silages was via the direct addition of chemical additives (e.g., antifungal compounds like propionic acid). In fact, addition of homolactic acid bacteria alone to accomplish this goal was not consistent because such fermentations often produced inadequate amounts of anti-fungal compounds, allowing for the accumulation of lactate assimilating yeasts that sometimes made aerobic stability worse than if the silage had not been treated. However, in a paradigm shift, Muck (1996) identified *Lentilactobacillus buchneri*, a heterofermentative lactic acid bacteria, as a potential bacterial inoculant because it could anaerobically convert moderate amounts of lactic to acetic acid. Acetic acid being highly antifungal, resulted in improved aerobic stabilities. One of the limitations of using *L. buchneri* has been that it was a relatively slow growing organism, and improvements in stability were usually observed only after more than 60-75 d of ensiling. Recent commercial inoculants have been developed that have combined *L. buchneri* with other bacteria from its family (e.g., *L. hilgardii* [da Silva et al., 2020] and *L. diolivorans* [Moyer et al., 2019]) resulting in more rapid improvements in aerobic stability as early as 10-30 d of ensiling. Recent research has also shown that bacterial inoculants can produce “bio active” compounds that could beneficially change the microbiome of silages.

The following is a brief list of some future potential target areas for developing bacterial inoculants to improve silage quality:

- Consistent improvements in ruminal fiber digestion of silages (e.g., targeting linkages that are not usually active in the rumen, being especially for low quality forages).

- Further improvements in aerobic stability could be realized if organisms were identified that could grow even more quickly

- Bacterial production of proteases (rather than exogenous addition) that could affect the prolamin: starch matrix to improve ruminal starch digestion of maize crop silages

- Inoculations that could improve the use of silages as biofuels

- Inoculation resulting in lower greenhouse gas emissions

- Inoculant formulations capable of improving silage fermentations under extreme environmental conditions (e.g., heat and cold)

- Inoculation to prevent the production of mycotoxin formation in the silo and to detoxify mycotoxins brought in from the field

- Identifying mixed formulations of bacterial inoculants with other exogenous additives (e.g. enzymes and chemical additives for synergistic effects on overall silage quality

- Use of a multi-omics approach to identify new strains of beneficial bacteria producing bioactive compounds (e.g., lysine, B vitamins, enzymes, iso acids, bacteriocins, etc.) that have production and health benefits to ruminants

Future research should also identify ways to improve the properties of bacterial silage inoculant formulations. For example, in many areas of the world, refrigeration of inoculants is impractical. Some specific target areas for improvements to the formulation of bacterial inoculants are listed:

Improvements in product shelf life (e.g., use of spore forming bacilli)

Better viability under extreme conditions in the silo (e.g., wider temperature- and osmo-tolerance)

Better heat and cold tolerance of formulations in liquid applicator tanks

Conclusions

The future of bacterial inoculants for improving silage quality is bright but it is difficult to pinpoint what benefits the next successful inoculants will bring.

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***In vitro* Rumen Fermentation of Grass, Clover and Corn Silage-based Diets with Different Protein Sources**

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Keywords: Grass silage, red clover silage, maize silage, protein feed

Introduction

The nutritive characteristics of different forage types differ and may influence the production response of diets. Understanding how different protein feeds interact with various forage sources helps in formulating diets that optimize nutrient utilization by the ruminant. For grass silage, the mixture of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) is typically used in the boreal growing conditions of Finland due to its winter hardiness, nutritive quality, and re-growth capability. Red clover (*Trifolium pratense*) is the most important forage legume in Northern Europe. Owing to its capability to fix atmospheric nitrogen, interest in growing red clover has increased due to high nitrogen fertilizer costs. Typically, red clover contains more crude protein (CP), but less neutral detergent fiber (NDF) than grass silage (Vanhatalo et al., 2009). The substitution of grass silage with red clover silage has increased total volatile fatty acid (VFA) concentration and proportion of acetate in the rumen and decreased the proportion of propionate and butyrate (Vanhatalo et al., 2009). Recently, the cultivation of forage corn has gained increasing interest in southern parts of Finland due to climate change. Compared to grass silage, corn silage is lower in CP and contains starch, which typically favors propionate production over acetate and butyrate production in the rumen (Giagnoni et al., 2024). Rapeseed cake and meal are the most important protein feeds for lactating dairy cows in Finland, but the cultivation areas have declined e.g., due to challenges related to insect pest management. New protein feeds like camelina (*Camelina sativa*) or hempseed (*Cannabis sativa*) cake are promising alternatives to rapeseed, as they diversify crop rotations and are rich in CP and unsaturated fat. The objective of this experiment was to study the impacts of rapeseed, camelina or hemp cake on *in vitro* rumen fermentation characteristics of grass, red clover or corn silage-based diets.

Materials and methods

The *in vitro* experiment had 12 treatments consisting of four protein feed options (negative control without protein feed, rapeseed cake, camelina cake or hemp cake) and three forage options (1st cut grass silage, 1st cut red clover silage or corn silage). Treatments also contained barley and the forage:concentrate ratio of all treatments was 1:1 on dry matter (DM) basis. Rumen inoculum was collected from two fistulated Finnish Ayrshire cows. The ratio of filtered (250 µm) rumen liquid to McDougall buffer was 1:2 (v:v). The determination of VFA was carried out at 1, 4, 8, 12, 24 and 30h from the beginning of the experiment. The experiment was repeated four times with Gas Endeavour *in vitro* system (Bioprocess control, Lund, Sweden). The CP concentration of negative control diets ranged from 95 to 140 g/kg DM depending on the forage source. For protein supplemented treatments, the CP concentration of diets was 170 g/kg DM. Experimental data was subjected to analysis of variance for repeated measures using the MIXED procedure of SAS (version 9.4).

Results

Grass, red clover, and corn silage had CP concentrations of 133, 156 and 65 g/kg DM, NDF concentrations of 518, 363 and 397 g/kg DM, and digestible organic matter in DM (D-value) of 710, 626 and 679 g/kg, respectively. The starch concentration of corn silage was 280 g/kg DM. Rapeseed, camelina and hemp cake had CP concentration of 332, 267 and 286 g/kg DM and NDF concentration of 194, 211 and 482 g/kg DM. Red clover silage-based diets resulted in highest molar proportion of acetate, lowest proportion of butyrate, and higher total VFA concentration than corn silage-based diets ($P<0.05$; Figure 1). Molar proportion of propionate was higher for corn silage than for grass silage ($P<0.05$), likely due to starch in corn silage. Protein supplementation had no impact on VFA production. Camelina resulted in higher ($P<0.05$) molar proportion of propionate and lower ($P<0.05$) proportion of butyrate than rapeseed cake. Compared to rapeseed and camelina, hemp cake resulted in highest proportion of acetate ($P<0.05$) and lower total VFA production ($P<0.05$) and molar proportion of propionate ($P<0.05$) than camelina. Only a few interactions were observed between dietary forage and protein source. On grass silage-based diets camelina tended ($P<0.10$) to result in higher total VFA production than rapeseed cake; the opposite was true for red clover silage-based diets.

Conclusions

Both forage and protein source influenced VFA production *in vitro*, but only a few interactions were observed between these factors. This indicates that their effects on ruminal VFA production might be independent of each other and the effect of the studied forage sources are potentially replicable *in vitro* irrespective of the type of protein supplementation used in the diet.

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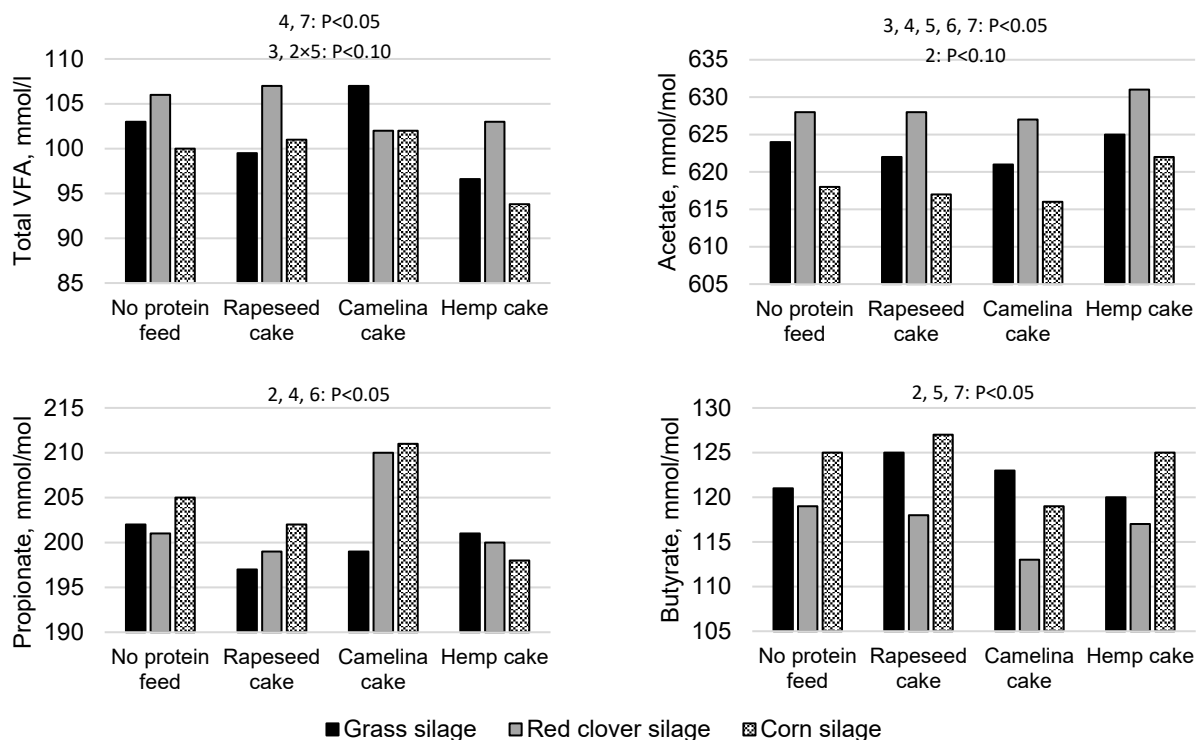


Figure 1.
The effect
of forage
source and
protein

supplementation on ruminal VFA production *in vitro*. Numbers in the figure refer to orthogonal contrasts with statistically significant difference. Contrasts tested: 1 = Effect of protein supplementation, 2 = camelina vs. rapeseed, 3 = hemp vs. rapeseed, 4 = camelina vs. hemp, 5 = red clover silage vs. grass silage, 6 = corn silage vs. grass silage, 7 = corn silage vs. red clover silage, 2×5, 2×6, 3×5 and 3×6. SEM for total VFA 3.14, for acetate 0.827, for propionate 2.87, and for butyrate 2.17.

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Ensiling Moist Cereal–legume Intercrops: A Promising Conservation Technique, Reducing Anti-nutritional Factors in Faba Beans

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Keywords: intercrop, cereal, legume, silage, anti-nutritional factors

Introduction

Sustainable agriculture is a hot topic in which self-sufficiency for protein sources in animal feed plays an important role. In Europe, cereal-legume intercropping may be an interesting alternative for soybean imported from the Americas: e.g. pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) have nutritional properties that can compete with soybean, but their amino acid composition is sub-optimal. Combining a legume with a cereal crop can balance the amino acid composition (Auer *et al.*, 2023). To preserve cereal-legume grains, ensiling is a cost-effective conservation technique compared to toasting and expansion/extrusion. Since more profound insights into the silage properties of these crops harvested at dough stage are eligible, the fermentation characteristics of different cereal-legume intercrop combinations (barley-pea, triticale-faba bean and wheat-faba bean) were studied, evaluating the effect of different silage additives. Furthermore, the effect of the ensiling process on the anti-nutritional factors (ANFs) vicine and convicine present in faba beans was investigated.

Materials and methods

During the growing seasons 2020-2021 and 2022-2023, intercrop trials were laid out at the Research Farm Bottelare in Belgium as described by Landschoot *et al.*, 2024. A selection of 15 intercrops was threshed at the hard dough stage, milled to 3-5 mm particles and treated with different silage additives: In both 2021 and 2023, organic acids (propionic acid and formic acid 1:1 at 6 l ton⁻¹ fresh matter (FM)) and Magniva Platinum Wholecrop (2 gram ton⁻¹ FM; Lallemand sas, France) were compared to a negative control treated with water; in 2021 and 2023, resp. Magniva Classic+ or Magniva Platinum 3 (2 gram ton⁻¹ FM; Lallemand sas, France) was included as third additive. Four replicates per treatment were ensiled in microsilos with a volume of 2.75 liter. At silo opening after 90 days, the silage fermentation characteristics were determined: dry matter (DM) content, crude protein (CP), ammonia fraction (NH₃-N/total N), pH, and alcohols (Alc.) were determined per replicate. Lactic acid (LA), acetic acid (AA), propionic acid (PA) and butyric acid (BA) were determined on mixed samples per treatment. Analyses methods were as described in Wambacq *et al.*, 2013. For the intercrops containing faba beans, vicine (VIC) and convicine (CON) were also quantified pre- and post-ensiling (LC-PDA-HRMS method based on Purves *et al.*, 2018).

Results

The fermentation characteristics and the reduction in vicine and convicine (for faba bean silages) are presented in Table 1, not per individual intercrop but per crop combination. BA was below the limit of detection in all samples, as was PA except for the acid-treated silages. Therefore, BA and PA are not mentioned in the table. Wheat-faba bean silages showed the highest crude protein levels followed by triticale-faba bean silages and barley-pea silages, highlighting the superior nutritional value of the faba bean containing silages. The control silage showed a good acidification in combination with a low ammonia fraction (McDonald *et al.*, 1991), and the different silage additives clearly influenced the fermentation characteristics. Acid treatment hampered the activity of epiphytic micro-organisms, resulting in a low ammonia fraction and low levels of LA, AA and Alc. The inoculants stimulated the fermentation process, resulting in a reduced pH and increased LA levels compared to the control; the AA and Alc levels differed in function of the inoculant composition. A very strong reduction of VIC and CON levels (resp. 76-100% and 62-100% compared to the levels pre-ensiling) was observed for all treatments, with significant differences between treatments for both ANFs in the intercrop wheat-faba bean and for CON in triticale-faba bean. This reduction facilitates the inclusion of faba beans in the ration of monogastric animals.

Table 1. Effect of silage additive treatment on the fermentation characteristics and reduction of anti-nutritional factors in the intercrops barley-pea (B-P), triticale-faba bean (T-Fb) and wheat-faba bean (W-Fb): dry matter content (DM), crude protein content (CP), NH₃-N/total N (Amm.fr.), pH, lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acids (BA), alcohol (Alc.), and reduction of vicine (VIC) and convicine (CON) compared to levels pre-ensiling. Different letters point to significant differences (Dunn test) between the additives per intercrop.

Inter- crop	Additive	DM ----- g kg ⁻¹ DM -----	CP ----- g kg ⁻¹ DM -----	Amm.fr. ----- Ratio -----	pH	LA	AA	Alc	VIC	CON g kg ⁻¹ FM	g kg ⁻¹ DM	Ratio
B-P	Control		712c	162a	3.17		4.80a	16.0d	4.37b	5.68a		
	Acids		720a	156bc	2.00		4.60b	0.22e	0.73d	0.60c		
	MPWC		710bc	166a	2.86		4.27c	27.9b	12.7a	6.04a		
	MP3		692d	143c	2.93		4.13d	21.8c	10.2a	3.57ab		
	MC+		720ab	159ab	3.13		4.22d	35.2a	3.26c	4.07b		
T-Fb	Control		708a	187a	2.63c		4.67a	13.4c	3.79c	3.36b	92	80a
	Acids		711a	183ab	1.83d		4.72a	0.41d	0.46e	1.36c	92	74ab
	MPWC		698c	189a	3.81b		4.21c	19.4b	7.37b	4.89a	86	66bc
	MP3		702bc	177b	4.42a		4.17d	24.5a	8.22a	4.12a	89	67bc
	MC+		705ab	190a	2.81c		4.39b	14.1c	1.79d	4.72a	86	62c
W-Fb	Control		691bc	255b	2.48a		4.73a	14.4d	3.69b	4.00b	88ab	91b
	Acids		705ab	255ab	0.97c		4.86a	1.51e	0.45d	1.95c	79b	81c
	MPWC		703ab	222b	2.57a		4.30c	19.4c	10.1a	7.03a	97a	97a
	MP3		690c	318a	1.80b		4.35bc	27.1a	9.70a	4.58ab	100a	100a
	MC+		710a	219b	2.10ab		4.39b	20.7b	2.14c	5.72ab	76b	70d

Control: water; Acids: propionic acid and formic acid 1:1 at 6 l ton⁻¹ fresh matter (FM); MPWC, MP3 and MC+: Magniva Platinum Wholecrop, Magniva Platinum 3 and Magniva Classic+ at 2 g ton⁻¹ FM (Lallemand sas).

Conclusions

From this study it can be concluded that the cereal-legume intercrops barley-pea, triticale-faba bean and wheat-faba bean harvested at hard dough stage can be converted into a qualitative feed source by ensiling. Despite the high DM and CP contents, which might hamper the ensiling process, the silage quality is good even without application of an additive. Except for protein content, the effect of intercrop combination was subordinate to the effect of silage additives. However, additives can steer the fermentation process into the desired direction. An additional benefit when faba beans are ensiled is the strong reduction of VIC and CON, rendering the silage suitable as both ruminant and monogastric feed.

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Effect of *Lactocaseibacillus huelsenbergensis* DSM 115424 on Fermentation of Grass and Corn Silage at Different Storage Temperatures

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Keywords *Lactocaseibacillus huelsenbergensis*, fermentation, storage temperature

Introduction

Climate change has been noticeable in agriculture for several years, presenting new challenges in the form of extreme weather conditions such as hot summers and periods of drought or flooding. For forage production, a hot summer means that the crop is harvested at high ambient temperatures. Silage inoculants based on lactic acid bacteria (LAB) are used to optimize the overall conservation process and thus silage quality (Kung et al, 1993). The favourable temperature for LAB growth and multiplication in a silage is 30-35°C, thus unfavourable living conditions such as high temperatures prevent optimal fermentation. A specially selected LAB strain named *Lactocaseibacillus huelsenbergensis*, isolated from an Austrian grass silage at 45°C, was published as a new species of the genus *Lactocaseibacillus* in 2023 (Grabner et al., 2023).

The aim of the present study was to investigate the effect of *L. huelsenbergensis* DSM 115424 on lactic acid production and pH reduction in corn and grass silages at two different storage temperatures (20°C and 40°C).

Material and methods

Fresh chopped corn plants (28.6% DM, 10.4% WSC, 28.0% starch, log 5.2 CFU LAB/ g FM, log 6.0 CFU yeasts/ g FM and log 4.3 CFU molds/ g FM) and permanent grassland dominated by Tall fescue (22.4% DM, 13.2% CA, 22.1% CP, 8.5% WSCs, log 5.7 LAB/ g FM, log 4.0 yeasts/ g FM, log 4.0 CFU molds/ g FM) were ensiled in triplicate for 3, 7 and 90 days - in laboratory mini silos (1500mL). Treatments consisted of an untreated control (CON) and treated with a homofermentative LAB strain (LH: *L. huelsenbergensis* DSM 115424); application rate: 250,000 CFU/ g FM). The mini silos were stored constantly at 20°C and 40°C. Measured parameters included: chemical composition of the fresh and ensiled corn and grass. Fermentation weight losses were determined after 90 days of storage based on the mass difference of the mini silos before and after ensiling and expressed as percentage fresh matter (Weissbach, 1998). Silage DM were corrected for losses of ethanol, PG, volatile fatty acids, and lactic acid due to drying (Weissbach and Kuhla, 1995). Concentration of fermentation acids, ethanol and PG from each mini silo was measured by high performance liquid chromatography (HPLC). Yeasts counts were determined on YGC agar. Lactic acid bacteria counts were determined on MRS agar.

For statistical evaluation, means and standard deviations were calculated for each parameter. The data were examined by SAS evaluation including Kruskal-Wallis test for significant differences ($P < 0.05$) between the control and the treatment group.

Results

Generally, lactic acid production was higher in both treatments at 40°C storage temperature compared to 20°C storage temperature for corn and grass silage at all opening days (Table 1 and 2). One exception is the grass silage at opening day 90, where the lactic acid content in CON at 40°C is lower compared to 20°C. Addition of LH resulted in a greater decrease in pH after storage at 20°C and 40°C on all opening days ($P < 0.001$) and was valid for both corn and grass silages, resulting in higher concentrations of lactic acid compared to CON. At 40°C storage temperature butyric acid was observed in CON at day 3, which continued to increase with longer storage time (day 7 and day 90) ($P < 0.001$). Fermentation weight losses were higher at 40°C storage temperature compared to 20°C for corn and grass silage and for both treatments. However, the untreated corn and grass silages showed higher weight losses in contrast to LH ($P < 0.001$) at both storage temperatures. It is assumed that the higher storage temperature has supported the growth of undesirable bacteria especially in CON silages due to higher pH and lack of competitive strength of the inoculated LAB. Yeast count was reduced faster with using LH compared to CON and in general, the addition of LH resulted in higher LAB counts at all opening days (exception: day 90 at 40°C for grass silage). Thus, the results of this study indicate the potential of *L. huelsenbergensis* DSM 115424 to improve silage quality and fermentation weight loss at different storage temperatures without showing decreased activity at the warm temperature of 40°C - which was expected due to the isolation of this novel species from grass silage at 45°C (Grabner et al., 2023).

Table 1. Effect of treatment (CON or LH) on dry matter (DM), pH, concentrations of organic acids and alcohols (g/ kg DM), lactic acid bacteria (LAB, log₁₀cfu/g), yeasts (log₁₀cfu/g) and weight losses (WL, % FM) of corn ensiled for 3, 7 and 90 d.

Day Treatment	DM _c	pH	LA	AA	BA	PD	Eth.	LAB	Yeast	WL
D3 CON20	31.9	4.25	18.2	6.4	0.0	0.0	4.7	8.1	6.2	
D3 LH20	32.1*	4.15*	20.6*	6.3	0.0	0.0	5.0	8.4*	6.0	
D3 CON40	32.8	4.06	19.9	7.0	0.0	0.0	3.1	8.3	4.8	
D3 LH40	32.3*	3.93*	30.7*	7.0	0.0	0.0	4.5	8.8*	4.7	
D7 CON20	31.8	3.87	31.2	7.7	0.0	0.0	9.8	8.5	5.3	
D7 LH20	32.2*	3.62*	42.4*	6.8*	0.0	0.0	10.5	9.0*	4.6*	
D7 CON40	31.9	3.90	35.7	7.8	0.0	0.0	7.5	6.9	5.4	
D7 LH40	32.6*	3.57*	52.1*	7.3	0.0	0.0	8.1	9.0*	4.0*	
D90 CON20	31.2	3.83	45.7	10.5	0.0	1.4	19.6	7.9	3.5	1.3
D90 LH20	31.8*	3.74*	49.8*	10.4	0.0	1.6	20.0	8.8*	<2.0*	0.8*
D90 CON40	31.8	3.76	52.6	17.6	0.0	0.8	20.4	<4.0	<2.0	1.5
D90 LH40	32.3*	3.64*	67.7*	11.3*	0.0	0.6	12.1	4.2*	<2.0	1.0*

CON20/ LH20 = CON/ LH at 20°C storage temperature; CON40/ LH40 = CON/ LH at 40°C storage temperature; *symbolize significant differences (P<0.05)

Table 2. Effect of treatment (CON or LH) on dry matter (DM), pH, concentrations of organic acids and alcohols (g/ kg DM), lactic acid bacteria (LAB, log₁₀cfu/g), yeasts (log₁₀cfu/g) and weight losses (WL, % FM) of grass ensiled for 3, 7 and 90 d.

Day Treatment	DM _c	pH	LA	AA	BA	PD	Eth.	LAB	Yeast	WL
D3 CON20	22.1	4.68	35.5	8.2	0.0	0.0	1.0	7.5	4.7	
D3 LH20	23.3*	4.42*	44.9*	9.1	0.0	0.0	1.0	8.7*	3.6	
D3 CON40	22.4	4.61	43.0	8.3	1.6	0.0	4.4	7.9	4.9	
D3 LH40	23.0*	4.29*	52.3*	8.3	0.0*	0.0	4.2	8.8*	3.6	
D7 CON20	22.2	4.45	46.9	11.6	0.0	1.8	1.8	8.3	3.9	
D7 LH20	22.7*	4.08*	68.6*	11.3	0.0	2.0	1.9	8.9*	2.8*	
D7 CON40	22.3	4.40	51.3	10.2	2.4	0.0	25.2	5.9	4.7	
D7 LH40	23.1*	4.10*	77.9*	10.7	0.0*	0.0	24.2	7.9*	3.7*	
D90 CON20	22.0	4.25	63.8	12.6	0.9	2.5	4.0	8.1	<2.0	1.9
D90 LH20	22.2*	4.05*	75.1*	13.8	0.0*	2.6	4.0	8.6*	<2.0	0.9*
D90 CON40	21.3	4.55	56.8	14.5	13.4	1.1	28.4	5.4	<2.0	2.7
D90 LH40	22.3*	4.08*	77.7*	10.4*	0.0*	0.8	23.4*	5.0*	<2.0	1.1*

CON20/ LH20 = CON/ LH at 20°C storage temperature; CON40/ LH40 = CON/ LH at 40°C storage temperature; *symbolize significant differences (P<0.05)

Conclusion

The results of this study indicate the potential of *L. huelsenbergensis* DSM 115424 to improve silage quality and fermentation weight loss at warm storage temperatures. The use of LAB mixtures containing *L. huelsenbergensis* DSM 115424 is recommended to farmers especially when high storage temperatures are expected.

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Effect of a Mixture of Homofermentative Lactic Acid Bacteria on the Fermentation Quality of Liquid Feed Containing Pea and Lupin Meal

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Keywords: fermentation, lactic acid bacteria, liquid feed, lupins, peas

Introduction A common approach to improve the hygienic status of liquid feed for pigs and extend its shelf life, is controlled fermentation. In controlled fermentation specific microorganisms, most commonly lactic acid bacteria (LAB), are used as inoculum to reduce the substrate pH rapidly. In contrast, uncontrolled fermentation is a spontaneous process, carried out only by naturally occurring, mostly epiphytic microorganisms (Canibe and Jensen, 2012). In recent years, pulses (beans, peas, lupins) are more frequently included in pig diets in Europe, as strategy to lower the carbon footprint of production and dependency on imported protein sources. However, when included into liquid feed, ground pulses are more difficult to acidify, due to their high protein content and buffering capacity. Particularly liquid feed containing a high percentage of beans, peas or lupins could potentially benefit from inoculation and controlled fermentation. This study was designed to investigate the fermentation kinetics of pea and lupin meal in response to inoculation with a mixture of homofermentative LAB.

Material and Methods This study consisted of two in vitro experiments. Experiment 1 compared the fermentation profiles and pH of ground peas (216 g crude protein [CP]/kg dry matter [DM]) and lupins (400 g CP/kg DM) to wheat (122 g CP/kg DM). All three substrates were fermented with (adLAB) and without (CON) a homofermentative inoculum. The inoculum consisted of equal parts of three homofermentative LAB strains (*Lactobacillus paracasei*, *Pediococcus pentosaceus*, and *Lactobacillus rhamnosus*; Lactosan GmbH and Co. KG, Kapfenberg, Austria) and was added at 2×10^5 CFU/g liquid feed (wet wt.). Experiment 2 was designed to compare the pH and fermentation profiles of basic rations, containing wheat and peas or wheat and lupins, with (adLAB) and without (CON) the same inoculum used in experiment 1. The liquid rations were composed of 716 g H₂O + 170 g wheat + 114 g peas (Pea mix) or 716 g H₂O + 170 g wheat + 114 g lupin (Lupin mix). For both experiments, the dry feeds were ground using a hammer mill before mixing. For each treatment, substrates were fermented in four replicates (1L screw-top vessels) at 37 °C for 24 h. Initial (0 h) and final pH (24 h) were recorded. Fermentation products were quantified by HPLC. Data were analyzed using the MIXED procedure of SAS (Version 9.4). The model included the fixed effects of substrate, treatment and their interaction. Fermentation vessel was considered as random effect. Treatment means were compared by Tukey-Kramer test. Threshold level for significance was $P < 0.05$.

Results

Table 1. Substrate pH and fermentation end products (g kg/DM) of wheat, peas, and lupins fermented without (CON) and with homofermentative lactic acid bacteria (adLAB) for 24 h (n=4).

Item ¹	Wheat		Peas		Lupins		SEM	Sub ⁵	P-values ⁴	
	CON ²	adLAB ³	CON	adLAB	CON	adLAB			Trt ⁶	Sub×Trt ⁷
pH 0h	6.18 ^a	6.18 ^a	6.05 ^b	6.05 ^b	5.63 ^c	5.64 ^c	0.022	<0.001	0.820	0.960
pH 24h	4.30 ^b	3.73 ^d	4.78 ^a	4.13 ^c	4.82 ^a	4.36 ^b	0.031	<0.001	<0.001	0.028
pH decline ⁸	1.88 ^b	2.46 ^a	1.28 ^c	1.93 ^b	0.81 ^d	1.28 ^c	0.030	<0.001	<0.001	0.030
Lactic acid	18.5 ^d	32.1 ^c	18.0 ^d	49.4 ^b	40.6 ^{cb}	61.0 ^a	2.00	<0.001	<0.001	0.001
Acetic acid	7.88 ^b	1.60 ^d	14.31 ^a	1.08 ^d	5.91 ^{cb}	2.40 ^{cd}	0.878	0.002	<0.001	<0.001
Butyric acid	6.85 ^b	1.02 ^c	19.64 ^a	0.04 ^c	0.04 ^c	0.04 ^c	0.524	<0.001	<0.001	<0.001
Ethanol	6.39 ^{ba}	1.13 ^b	7.05 ^a	4.26 ^{ba}	3.05 ^{ba}	0.82 ^b	1.310	0.036	0.005	0.482

¹Concentrations of 1,2-propanediol, n-propanol, and propionic acid were below the detection limit (<0.001 g/kg DM) in all fermentation vessels.

²Control

³All adLAB treatments were supplemented with a mixture of *Lactobacillus paracasei*, *Pediococcus pentosaceus*, *Lactobacillus rhamnosus* (Lactosan GmbH and Co. KG, Kapfenberg, Austria) at 2×10^5 CFU/g liquid feed (wet wt.)

⁴Within a row, means without a common superscript letter differ, $P < 0.05$.

⁵Substrate (Sub) = ground wheat, peas or lupins; ⁶Treatment (Trt) = control (CON) or adLAB; ⁷Interaction of substrate × treatment

⁸pH decline = pH 0h – pH 24h

Addition of LAB resulted in a greater decline in pH compared to CON (Table 1) for all three substrates ($P < 0.001$). Overall, the pH decline was the highest for wheat adLAB, followed by lupin adLAB, and pea adLAB. Across all treatments, adLAB resulted in higher concentrations of lactic acid compared to CON ($P < 0.001$), whereas concentrations of acetic acid and ethanol in the CON treatments were substantially higher compared to adLAB ($P < 0.001$). The addition of LAB to wheat and especially peas also resulted in much lower concentrations of butyric acid compared to wheat CON and peas CON ($P < 0.001$), while there was no difference in butyric acid between CON and adLAB lupins.

In experiment 2, results were similar to experiment 1 and foremost affected by LAB supplementation (Table 2). Substrate pH (24h) of adLAB were lower compared to CON in both treatments ($P < 0.001$). Lactic acid concentrations in response to adLAB were higher compared to CON ($P < 0.001$). The addition of LAB as well resulted in lower concentrations of acetic acid, butyric acid, and ethanol compared to CON in the lupin, as well as the pea mix ($P < 0.001$).

Table 2. Crude protein, substrate pH and fermentation end products (g kg¹DM) of mixed substrate containing peas and lupins fermented without (CON) and with homofermentative lactic acid bacteria (adLAB) for 24 h (n=4).

Item ¹	Pea mix		Lupin mix		SEM	P-values ⁴		Sub × Trt ⁷
	CON ²	adLAB ³	CON	adLAB		Sub ⁵	Trt ⁶	
Crude protein (0h)	208	208	313	313				
pH (0h)	6.03 ^a	6.03 ^a	5.85 ^b	5.88 ^b	0.028	<0.001	0.547	0.666
pH (24h)	4.57 ^b	3.87 ^c	4.76 ^a	3.90 ^c	0.017	<0.001	<0.001	0.000
pH decline ⁸	1.46 ^c	2.16 ^a	1.13 ^d	1.95 ^b	0.033	<0.001	<0.001	0.071
Lactic acid	9.91 ^c	44.36 ^b	10.76 ^c	55.82 ^a	1.091	0.000	<0.001	0.000
Acetic acid	10.84 ^a	0.88 ^b	11.34 ^a	1.55 ^b	0.546	0.304	<0.001	0.878
Butyric acid	13.90 ^a	0.04 ^b	15.28 ^a	0.70 ^b	0.883	0.270	<0.001	0.690
Ethanol	6.76 ^a	1.24 ^c	2.52 ^b	0.88 ^c	0.141	<0.001	<0.001	<0.001

¹Concentrations of 1,2-propanediol, n-propanol, and propionic acid were below the detection limit (<0.001 g/kg DM) in all fermentation vessels.

²Control

³Both adLAB treatments were supplemented with a mixture of *Lactobacillus paracasei*, *Pediococcus pentosaceus*, *Lactobacillus rhamnosus* (Lactosan GmbH and Co. KG, Kapfenberg, Austria) at 2×10^5 CFU/g liquid feed (wet wt.)

⁴Within a row, means without a common superscript letter differ, $P < 0.05$.

⁵Substrate (Sub) = pea mix and lupin mix; ⁶Treatment (Trt) = control (CON) or adLAB; ⁷Interaction of substrate × treatment

⁸pH decline = pH 0h – pH 24h

Conclusion

This study confirms that adding selected homofermentative LAB is beneficial, particularly when liquid feeds with high CP content and buffering capacity, are fermented. Substrate pH of <4.5, which is the common threshold to suppress undesired microorganisms such as enteropathogenic *E. coli*, *Salmonella*, and *Klebsiella* (Scholten et al., 1999), was only achieved by LAB supplementation. It should be emphasized, that our data were generated in laboratory-scale batch experiments. Practical feeding conditions are far less ideal and can be impacted by a multitude of adverse factors, therefore the response to inoculation treatments can be more variable in practical farming.

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Effect of Corn Silage Hybrid Characteristics and Fermentation on Key Forage Quality Metrics

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Keywords: corn silage, corn silage processing score, ear to stover ratio, fermentation

Introduction

Corn silage harvest management has a significant role in optimizing the value of this feedstuff for ruminants as there are opportunities to enhance, maintain, or diminish the value of the forage through the harvesting process. Corn kernels contain a significant fraction of the total starch in corn silage and while starch content and digestibility are routinely measured, starch availability is dependent on the processing (breaking) of the corn kernels to expose the contents of the kernel to microbial activity in the digestive track.

Adequate kernel processing can be determined by evaluating the corn silage processing score (**CSPS**), which measures the starch particle size. It has been hypothesized that the performance of the harvester processor unit could be influenced by plant characteristics, but the magnitude of this influence is not well understood. Current industry guidelines categorize CSPS as follows; a score below 50 is “inadequate”, a score of 50 to 70 is “adequate” and a score greater than 70 is “optimum”.

The objectives were to 1) evaluate the effects of hybrid characteristics on CSPS at harvest and 2) evaluate if fermentation alters in vitro starch digestibility (**IVSD**; 7 hr, 4 mm), neutral detergent fiber after 30 h of fermentation (**NDFd30**, % of NDFom), and CSPS.

Materials and Methods

A two-year field study was conducted on dairy farms in New York State ($n = 5$ in year 1, $n = 3$ in year 2). To measure the impact of hybrid characteristics on CSPS, four commercially available corn hybrids exhibiting different characteristics (plant stature, relative maturity, kernel texture) were planted in field scale strips on each farm each year. Corn strips were planted by each host farm following agronomic best practices and monitored throughout the season.

Immediately prior to field scale harvest, 20 corn plants were randomly selected and hand harvested from each hybrid at a standardized height. Hand harvested subsamples were weighed as whole plants and then separated into the ear (kernels and cob) and stover (stalk, leaves, husk) components. The ear and stover samples were weighed and dried separately to determine DM concentration and ear to stover ratio.

For each site-year, prior to harvest of the field scale strips, the corn silage harvester was set up to meet industry standards for forage particle length and CSPS. The four field strips (hybrids) were then harvested on the same day by the field harvester at a standard ground speed and engine speed for a minimum of 240 meters. Four samples of chopped corn silage per hybrid were randomly collected after exiting the harvester spout for each hybrid (hybrid sample). For the same three locations per year (six site-years), the four hybrid samples were then split into four subsamples and vacuum-sealed to create mini-silos to evaluate how fermentation affects forage metrics, resulting in 64 vacuum sealed samples (4 hybrids x 4 chopped hybrid samples x 4 subsamples per chopped sample) per site-year. One subsample from each hybrid sample group was immediately submitted as a fresh unfermented sample to an accredited forage laboratory for analysis (standard NIR, IVSD, CSPS). The remaining three subsamples per hybrid sample were then placed in storage (at room temperature) to undergo fermentation for defined lengths of time (45, 90, and 135 d) and then submitted for the same laboratory analysis performed on the green samples.

All statistical analyses were conducted using SAS software (v.9.4). An ANCOVA (PROC MIXED) analysis was used to evaluate the effect of ear to stover ratio on CSPS for green samples and included hybrid, DM (whole plant or ear), and year as covariates and the random effect of site-year. A repeated-measures ANCOVA (PROC MIXED) with the repeated statement for fermentation timepoint and random effect of site-year was used to evaluate the effects of fermentation time on nutrient metrics. The models included the fixed effect of time and covariate effects of hybrid and year. *P*-values were corrected for multiple comparisons with a Tukey's honest significant difference test.

Results

Effect of Hybrid Characteristics on Corn Silage Processing Score at Harvest

Ear to stover ratio was associated ($P = 0.01$) with CSPS for green samples such that a 0.1-unit increase in ear to stover ratio resulted in a $0.73 (\pm 0.28)$ -percentage unit increase in CSPS. Hybrid was also significantly associated with CSPS ($P < 0.001$); however, whole plant DM ($P = 0.83$) and year ($P = 0.73$) were not. The analysis was repeated with ear DM replacing whole plant DM. When controlling for ear DM, ear to stover ratio was still associated ($P < 0.001$) with CSPS such that a 0.1-unit increase in ear to stover ratio resulted in a $1.7 (\pm 0.41)$ -percentage unit increase in CSPS. Ear DM was negatively associated with CSPS ($P = 0.01$) such that every 1-percentage unit increase in ear DM resulted in a $0.78 (\pm 0.30)$ -percentage unit decrease in CSPS. Given the range of ear to stover ratios (0.5 to 0.9) and ear DM (5 to 12 percentage units) between hybrids within a location, changes to these characteristics could have a meaningful impact on CSPS, requiring adjustments to the harvester processor unit for optimal processing.

Effect of Fermentation on Corn Silage Digestibility and Corn Silage Processing Score

Overall, we observed a positive effect of fermentation on IVSD (Table 1); however, NDFd30 was not affected (Table 1). The impacts of fermentation on whole plant digestibility observed in this study were consistent with previous studies (Ferraretto et al., 2014), where IVSD increased with fermentation time while fiber digestibility (NDFd30) was unchanged.

There was a tendency for fermentation time to be negatively associated with CSPS (Table 1); however, differences are minimal and may not be biologically significant. Our results contradict those of Ferraretto et al. (2015) and Saylor et al. (2020), who observed a positive association, whereas Agarussi et al. (2020) found a lack of response to fermentation in corn silage with very low CSPS scores.

Table 1. Least squares means and SE for the effect of fermentation time on IVSD, NDFd30, and CSPS.

Forage Metric	Fermentation Timepoint, d				SE	P-value
	0	45	90	135		
IVSD, % starch	58.2 ^d	63.2 ^c	67.2 ^b	69.4 ^a	1.7	< 0.001
NDFd30, % NDFom	57.4	57.1	57.0	56.8	0.8	0.23
CSPS, % starch	60.3 ^A	60.0 ^{BA}	59.3 ^{BA}	58.4 ^{BC}	5.9	0.07

^{a,b,c,d}Means with different superscripts differ ($P < 0.05$).

^{A,B,C}Means with different superscripts differ ($P < 0.10$).

Conclusions

The plant characteristics of ear to stover ratio and ear DM should be taken into consideration and adjustments to the harvester processor unit are warranted as these characteristics change during harvest season. Inadequate CSPS limits the ability of the animal to utilize starch, regardless of IVSD. The effect of fermentation on CSPS is inconsistent and should not be relied on to improve starch availability to the cow between harvest and feedout of corn silage.

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Lactic Acid Bacteria as a Silage Inoculant or Direct-fed Microbial and Effects on Rumen Metabolism

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Keywords: grass silage, fermentation, *Lactiplantibacillus plantarum*, *Pediococcus pentosaceus*

Introduction

Since the 1970's the inoculation of forage with lactic acid bacteria (LAB) to improve the retention of nutrients during fermentation has become commonplace. The dietary benefits of LAB inoculants to animal production have been well described (Muck et al. 2018). The production of fermentation end products during the ensiling process varies depending on several factors, including the physical and nutritional content of the plant at harvest, the inoculant strains used and the conditions during ensiling. Furthermore, management during the feed out period influences the quality of the silage and animal performance. The distinction between the contribution of the fermentation end-products produced during ensiling, or from the inoculant microbial biomass on rumen microbial metabolism has not been fully established. Studies that have been undertaken thus far have also mainly focused on rumen metabolism *in vitro*. This study aimed to distinguish the effects on rumen metabolism in dairy cows when LAB was used either as a silage inoculant or as a direct fed microbial (DFM).

Materials and methods

Grass (*Lolium perenne*) was harvested and ensiled with or without inoculant (*Lactiplantibacillus plantarum* IMI 507026 and *Pediococcus pentosaceus* IMI 507025 in a 50:50 mix) at an application rate of 10^6 CFU/g FM. Grass was compacted into two concrete walled clamps (~50 tonne), which were sealed and ensiled for approximately 28 weeks. The silages were analysed for their nutrient and fermentation profile and incorporated into a total mixed ration (TMR) containing 60:40 grass silage to concentrate ratio (DM basis) and fed to six rumen cannulated multiparous early lactation Holstein-Friesian cows in a 3 x 3 replicated Latin square design, with three periods each of 28 d duration. Dietary treatments were; TMR including silage ensiled without inoculant (CON), TMR including silage ensiled with inoculant (INOC), or CON supplemented with lyophilised LAB (consisting of a 50:50 mix of *L. plantarum* and *P. pentosaceus* at 4×10^{12} CFU/kg silage FM (DFM)). On day 25 of each period, at 08:00, 11:00, 14:00, 17:00, 20:00 h, rumen fluid samples were collected from the ventral region of the rumen for pH and volatile fatty acid (VFA) analysis. Data were analysed using Genstat (VSNi. v23) by either Student's t-test or by repeated measures analysis of variance (ANOVA). P-values of <0.05 were considered statistically significant.

Results

The lactic:acetic ratio of the INOC silage was higher than CON ($p=0.005$) (Table 1), although no difference in lactic acid concentration was observed between the silages. The inoculated silage contained a lower concentration of $\text{NH}_3\text{-N}$ ($p<0.001$), and acetic acid ($p<0.001$) compared with the control. Cows fed TMR containing INOC silage had a higher mean rumen pH of 6.20 than CON or DFM at pH 6.05 and 6.02, respectively ($p=0.031$) (Table 2) and there was no interaction ($p>0.05$) between sampling time and treatment. Treatment had no effect on the total rumen VFA (tVFA) concentration, or propionate, n-butyrate, iso-butyrate, n-valerate, iso-valerate as concentrations or as %tVFA, but there was a main effect of time. Mean rumen acetate concentration was increased in CON and DFM compared to INOC (55.0 mM, 51.3 mM and 55.9 mM for CON, INOC and DFM, respectively) with no main effect of time.

Table 1. Nutritive value and fermentation profile of grass silage ensiled without (Control) or with (Inoculated) lactic acid producing bacteria.

g/kg DM unless otherwise stated	Treatment			p-value* ²
	Control	Inoculated	sem	
DM	331	324	3.1	0.281
CP	133	136	1.3	0.206
NH ₃ -N* ¹	5.28	4.71	0.098	< 0.001
Ash	8.24	8.15	0.070	0.552
NDF	406	407	2.9	0.945
pH	3.91	3.89	0.016	0.630
Acetic acid	9.39	7.69	0.300	< 0.001
Lactic acid	93.3	95.0	1.20	0.549
Lactic:acetic	9.96	12.4	0.14	0.005
Ethanol	9.36	13.3	1.16	0.090
n-Butyric acid	1.86	1.82	0.192	0.926
Propane-1,2-diol	0.141	0.072	0.0196	0.075
Propionic acid	0.137	0.206	0.0368	0.392
Water soluble carbohydrates	104	102	3.6	0.777

*1. expressed as % of total N. *2. two tailed t-test.

Table 2. Rumen volatile fatty acid (mM) content of dairy cows fed a TMR comprising silage ensiled without inoculant (CON), TMR comprising silage ensiled with inoculant (INOC), or CON supplemented with lyophilised LAB (consisting of a 50:50 mix of *L. plantarum* and *P. pentosaceus*) (DFM).

	Treatment			SED	p-value		
	CON	INOC	DFM		Tr	Ti	Tr.Ti
Mean rumen pH	6.05	6.20	6.03	0.048	0.031	<.001	0.656
Total volatile fatty acids (mM)	93.5	87.1	94.7	2.48	0.055	0.003	0.926
Acetate (mM)	55.0	51.3	55.9	1.41	0.046	0.136	0.903
Propionate (mM)	22.2	18.9	21.8	1.65	0.193	<.001	0.835
Butyrate (mM)	12.7	13.4	13.1	1.65	0.929	<.001	0.948
Iso-Butyrate (mM)	0.709	0.686	0.758	0.0329	0.176	0.01	0.785
Valerate (mM)	1.86	1.76	1.85	0.071	0.35	<.001	0.881
Iso-valerate (mM)	1.06	1.08	1.28	0.155	0.355	<.001	0.899

Data was tested for normality using Shapiro Wilks normality test and subjected to repeated measures ANOVA.

Conclusions

The silage fermentation was altered by the addition of LAB with a lower ammonia-N and higher lactic:acetic ratio in inoculated silage. This also corresponded with a higher mean rumen pH in the INOC treatment compared to CON and DFM. This study determined that changes in rumen metabolism were associated with the indirect effects of LAB (silage fermentation end products or changes in the nutritive value of the silage), when added as an inoculum at ensiling and not the direct effect of LAB or their biomass.

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Amino Acids in Lucerne Silage: 1. Effects of Chop Length

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Keywords: lucerne, chop length, amino acids

Introduction

Lucerne has a low sugar content and high buffering capacity making the forage more difficult to ensile effectively. Silage quality is strongly influenced by the degree of protein degradation that occurs after harvest and during fermentation (Albrecht and Muck, 1991) and achieving a higher density of plant material during ensiling is one of the most significant factors limiting protein degradation (Fijałkowska *et al.* 2015). Here we investigated the effect of forage chop length at ensiling on the protein stability and amino acid profile of lucerne silage.

Materials and methods

A lucerne (*Medicago sativa*) crop was cut on 16 August 2022 (third harvest with 5 weeks growth) using a hand operated reciprocating blade mower. The forage was spread across the ground and allowed to wilt for 24 h. The forage was passed through a stationary forage harvester adjusted to cut to either 12 or 25mm theoretical chop length. Each forage was treated with a commercially available homofermentative *L. plantarum* inoculant NCIMB 40027 (Ecosyl 100, Volac Ltd, Royston, Herts., UK; 1×10^6 CFU g⁻¹) then ensiled for 96 days in 2 L glass silos packing the jars by hand. Due to the chop length, at a similar packing pressure, the silo density was higher for the short chop versus long chop treatment (1260 v 955 g fresh material, respectively). Samples of forage as mown and as ensiled and the resultant silages were freeze dried prior to determination of ash, water soluble carbohydrate (WSC), neutral-detergent fibre (NDF) nitrogen (N) and buffer soluble N content. Lactate, volatile fatty acid (VFA), pH and ethanol content was determined on frozen silage samples. Amino acid (AA) concentrations were determined in freeze-dried samples by Sciante Analytical Services Ltd (Berkshire, UK) using ion exchange chromatography.

Results

No differences were observed in composition or N solubility at ensiling (Table 1). Short chopping leading to higher density silos resulted in an improved fermentation (Table 2), as indicated by a lower silage pH and ethanol, and a higher lactate concentration ($P < 0.05$). There was a trend for lower N solubility due to shorter chop length ($P = 0.07$). There was no effect of chop length on total AA or essential AA concentration in the silages ($P > 0.05$) but the concentrations of free AA, both total and essential, was lower with the shorter chop length ($P < 0.001$) (Table 1). When free AAs were determined as a percentage of total AAs, a short chop length silage had lower free amino acids (57.0 v 62.0; s.e.m. 0.48; $P = 0.002$) and free essential AAs (33.4 v 38.1; s.e.m. 0.28; $P < 0.001$) compared to the long chop treatment silage. A short chop length also reduced the free essential AAs when determined as a percentage of the total essential AAs (66.1 v 74.1; s.e.m. 1.00; $P < 0.005$) when compared to long chop treatment.

Table 1. Composition of lucerne at ensiling

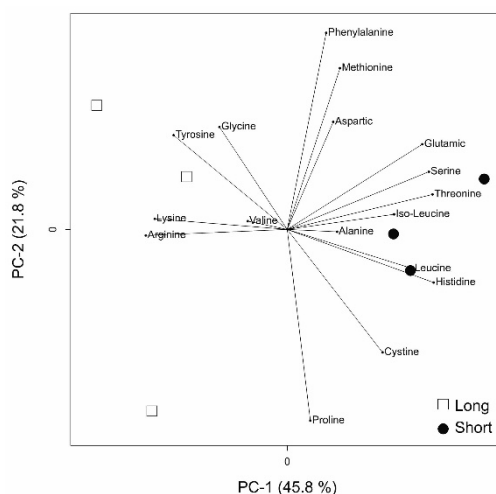
	Long	Short	s.e.m.*	Prob	
Freeze-dried DM (g/kg)	331.5	330.6	2.33	0.804	
Ash (g/kg DM)	99.6	97.2	1.71	0.366	
WSC (g/kg DM)	68.0	70.4	1.16	0.220	
NDF (g/kg DM)	412.9	406.6	9.27	0.654	
Total N (g/kg DM)	30.5	28.9	0.53	0.101	
Soluble N (g/kg TN)	478.8	438.9	16.01	0.153	* 3 subsamples

Effects of chop length on total concentrations (g/kg DM) of the individual AA's are demonstrated in the biplot in Fig 1. Principal component 1 accounted for 45.8% of the total variation with clear separation of the treatments on this axis. Based on analysis of variance of individual AA's (data not shown), the essential AA's threonine and histidine as well as non-essential serine and glutamic concentrations were higher in shorter chop silage ($P < 0.05$), whilst the essential AA's arginine and lysine total concentrations were lower in shorter chop ($P < 0.01$). Percentages of the essential AA, arginine and lysine as well as non-essential serine in free form were lower with shorter chop silage ($P < 0.001$) (Fig. 1). Percentages of essential AA threonine, leucine and histidine as well as non-essential aspartic, glutamic, alanine and proline in free form were higher with shorter chop ($P < 0.05$), with a similar trend shown for methionine ($P = 0.09$) (data not shown).

Table 2. Composition of lucerne at mowing and following ensiling for 90 days at two chop lengths.

	At mowing*		Silage**			
	Mean	s.e.m.	Long	Short	s.e.m.	Prob
pH	-	-	4.47	4.40	0.009	0.007
Lactate (g/kg DM)	-	-	68.2	79.6	1.30	0.003
Acetate (g/kg DM)	-	-	15.4	18.4	0.487	0.013
Ethanol (g/kg DM)	-	-	5.26	2.85	0.183	<0.001
Lactate (% TFA)	-	-	76.9	77.6	0.38	0.266
Total N (g/kg DM)	30.4	0.51	32.6	32.1	0.36	0.413
Soluble N (g/kg total N)	438.2	5.89	840.5	807.5	9.55	0.071
Amino acids (g/kg DM)						
Total	170.1 ¹	-	166.4	165.4	1.20	0.581
Total essential	83.3 ¹	-	85.7	83.6	1.17	0.270
Total Free	8.17 ¹	-	103.2	94.2	0.23	<0.001
Free essential	2.47 ¹	-	63.4	55.2	0.21	<0.001

* 3 subsamples; ** 3 jars per treatment; TFA - total fermentation acids ¹ single determination

**Figure 1.** Principal component (PC) biplot of total concentrations (g/kg DM) of amino acids in silages

Conclusions

A shorter chop length, accompanied by improved packing density, improved the fermentation, resulting in lower total concentrations of free AA compared to silage with a long chop length. Variable results were found for essential and non-essential AA total concentrations but when determined as a percentage of total AAs, a short chop length silage had lower free amino acids and free essential AAs compared to the long chop treatment silage.

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Lactic Acid Bacteria Inoculants as a Tool for Sustainable Forage Preservation and Rumen Fermentation Efficiency

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Keywords: Animal emissions; greenhouse gases; Livestock sustainability; rumen fermentation; silage preservation.

Introduction

Silage fermentation is a pivotal process in livestock production, enabling the preservation of forage crops to ensure consistent nutrient availability. This technique is critical in addressing seasonal forage scarcity and maintaining feed quality (Cheng *et al.*, 2022). However, the ensiling process is challenged by nutrient losses, the growth of undesirable microorganisms, aerobic spoilage, and its contribution to greenhouse gas (GHG) emissions. Lactic acid bacteria (LAB) inoculants have emerged as effective additives to improve silage quality with recent studies highlighting their ability to optimize fermentation dynamics, preserve dry matter (DM), enhance microbial stability and reduce methane production during ruminal digestion (Kim *et al.*, 2021; Wang *et al.*, 2022). This study investigated the effects of three lactic acid bacteria (LAB) inoculants—Ecosyl [*Lactiplantibacillus plantarum* MTD/1 (NCIMB 40027)], PJB1 [*Lentilactobacillus buchneri* PJB/1 (NCIMB 30139)], and Ecocool (a blend of *L. plantarum* with *L. buchneri*)—on the quality, microbial composition, and fermentation characteristics of perennial ryegrass (PRG)-based silages. Additionally, the impact of these inoculants on rumen fermentation efficiency was evaluated by measuring pH levels, gas production, VFAs levels, ammonia concentration, and methane emissions.

Materials and methods

The PRG was harvested, sieved to a size of 5–7 cm, and then treated with a suspension of LAB or sterile water as a control treatment (CON). The LAB treatments consisted of Ecosyl ($\sim 1 \times 10^6$ CFU/g), Ecocool ($\sim 3 \times 10^5$ CFU/g), and PJB1 ($\sim 2 \times 10^5$ CFU/g). Samples were stored in airtight jars at room temperature and analysed at 0, 4, 24, 48 hours, as well as 7, 14, 28, 90 days for pH, volatile fatty acids (VFAs), fresh weight change and microbial diversity. For 90 day-silage samples, *in vitro* incubations were performed in 100 ml serum bottles, with a 1:1 pooled rumen fluid from 4 animals and Van Soest buffer, at a constant temperature of 39°C. This incubation aimed to assess pressure, pH, methane production, ammonia concentration. Active bacterial communities were analyzed through full-length 16S rRNA gene sequencing using PacBio CCS technology, with the sequences processed using QIIME2. Any samples or species with ≤ 10 counts were excluded from the analysis. Taxonomic identities of amplicon sequence variants (ASVs) were assigned using the GreenGenes2 reference database. Alpha diversity was assessed using the Chao1, Shannon, and Simpson indices to evaluate species richness and evenness within the microbial communities. Beta diversity was analyzed using Bray-Curtis dissimilarity, with the results visualized through principal coordinates analysis (PCoA). Statistical significance of the beta diversity was determined using PERMANOVA and Wilcoxon rank-sum tests to compare differences between groups. All statistical analyses were performed using the R programming language.

Results

In the silage, Ecosyl and Ecocool resulted in the highest lactic acid concentrations ($P < 0.01$), significantly increasing its percentage in fermentation products relative to total VFAs, while preserving more DM than PJB1 and CON ($P < 0.05$). Both Ecosyl and Ecocool maintained significantly lower pH than PJB1 and CON throughout the study ($P < 0.05$). Significant weight changes were observed at 7, 28, and 90 days ($P < 0.05$), with Ecosyl and Ecocool exhibiting stable weight changes, indicating reduced gas losses and efficient ensiling fermentation. In contrast, PJB1 and CON showed moderate fluctuations, with CON displaying the greatest variability and weight loss. Furthermore, Ecosyl and Ecocool produced the highest levels of VFAs, particularly at 90 days ($P < 0.01$), with acetic acid being the most abundant. Ethanol production was significantly higher in PJB1 treatment at 90 days ($P < 0.05$), while CON exhibited lower levels of both VFAs and ethanol. Microbial profiles differed significantly between Ecosyl, PJB1, and the other treatments (CON and Ecocool). PCoA analysis revealed significant clustering by treatment and time ($P < 0.05$), suggesting that both factors notably influenced bacterial composition. Alpha diversity indices indicated that Ecosyl and PJB1 enhanced bacterial richness (Chao1) and diversity (Shannon, Simpson) compared to CON ($P < 0.05$). Microbial diversity increased early (at 14 days) but stabilized or declined in later stages (28–90 days). In the rumen, PJB1 reduced gas pressure ($P < 0.05$). Inoculation with PJB1 resulted in a significant reduction in methane compared to CON at 4 h (51.76%; $P < 0.05$), 24 h (64.96%; $P < 0.01$), and 48 h (51.62%; $P < 0.01$; Table 1), demonstrating the greatest reduction across time points. Ecocool also reduced methane levels but to a lesser extent 4 h (33.82%; $P < 0.05$), 24 h (23.39%; $P < 0.01$), and 48 h (48.47%; $P < 0.01$), highlighting its potential for mitigating greenhouse gas emissions.

Table 1: Data from Rumen *in vitro* fermentation of ensiled perennial ryegrass showing the data for 24- and 48-hour time points †. These comparisons have been made across all treatments against each other within each of time points.

Inoculant	pH		Ammonia (mM)		Methane (%)		pressure (psi)		Acetic (mg/ml)		Butyric (mg/ml)	
	24	48	24	48	24	48	24	48	24	48	24	48
Control	5.864a	5.502a	12.08a	16.41a	39.37a	55.55a	31.02a	36.64a	4260a	3706	860a	100
Ecosyl	5.914a	5.43a	8.83b	10.92b	34.04a	39.99ab	30.76a	36.7a	3908b	3310	878a	71
Ecocool	5.752a	5.314ab	7.68b	8.16b	30.15ab	28.62ab	29.56a	34.76a	4081ab	3393	991b	77
PJB1	6.21b	5.538ac	16.31c	18.07a	13.8b	26.87b	20.62b	29.82b	4136ab	3607	1016b	108

† Means within a column not followed by the same letter are significantly different ($P < 0.05$).

Conclusion

In conclusion, these findings underscore the potential of the evaluated LAB inoculants to enhance silage fermentation, nutrient retention, stabilize microbial community, and reduce methane emission. Such improvements contribute to sustainable livestock production by addressing key challenges in feed preservation and environmental impact.

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Effect of Forage Endogenous Cellulolytic Enzymes on the Degradation of Structural Carbohydrates

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Keywords: endogenous cellulolytic enzyme, structural carbohydrate, monosaccharide

Introduction

Ensiling, a technique that can effectively reduce nutrient loss, significantly enhance palatability, and enable long-term storage of forage, has been widely adopted in animal husbandry. Alfalfa, renowned for its high protein content, is the most important leguminous forage for ruminants. However, due to its low water-soluble carbohydrate content, obtaining quality silage from alfalfa poses a considerable challenge. To accelerate the degradation of structural carbohydrates and provide an ample substrate for lactic acid bacterial fermentation, it is imperative to comprehensively elucidate the degradation mechanism of structural carbohydrates during the ensiling process. Han and Chen (2010) suggested that plants constitute one of the important resources for cellulolytic enzyme production and successfully isolated and identified a β -exoglucanase from maize. Therefore, this study aimed to evaluate the influence of forage endogenous cellulolytic enzymes on the degradation of structural carbohydrates.

Materials and methods

Alfalfa was harvested at the early flowering stage and chopped into 1-2 cm lengths with a fodder chopper. The chopped alfalfa was loaded into vacuum bags (150g per bag), and then sterilized by electron-beam irradiation (32 kGy) to eliminate epiphytic microorganisms. The experiment followed a completely random design (6 ensiling days $\times$ 3 repetitions). The silos were opened on the 3, 6, 10, 15, 30 and 60 days after ensiling, respectively. Cellulose and hemicellulose were determined by the method of Van Soest et al. (1991). The monosaccharides and cellobiose were determined by High performance liquid chromatography. The cellulolytic enzyme activity was determined with the method of DNS (Deshavath et al., 2020). The data were analysed by one-way analysis of variance (SAS 9.4).

Results and Discussion

As shown in Table 1, the activities of CMCase, avicelase, β -glucosidase and FPase were 81.4, 30.5, 55.7 and 34.7 U/kg FW, respectively. Table 2 shows that the cellulose and hemicellulose contents have no significant ($P>0.05$) difference, whereas, the hemicellulose shows a continuous decreasing trend. This might be due to the fact that hemicellulose is easier to degrade compared with cellulose (Chen et al., 2019). The glucose content significantly increased ($P < 0.05$) during the first 15 days of ensiling. After 15 days of ensiling, the glucose content significantly ($P<0.05$) decreased, it was probably because the glucose is further degraded into other products (5-Hydroxymethylfurfural, acetic acid, propionic acid, etc.) (Lv et al., 2024). The mannose content reached its peak value at 10 days of ensiling, and galactose content reached its peak value at 15 days of ensiling. The cellobiose content continuously increased ($P<0.05$) during ensiling, which was contributed to by the activity of endoglucanase and exoglucanase. The endoglucanase could degrade cellulose into oligosaccharides, and then exoglucanase could degrade oligosaccharides into cellobiose.

Conclusions

Cellulose is degraded into oligosaccharides, cellobiose and glucose under the action of endogenous cellulolytic enzymes. The elevation in the content of mannose and galactose suggests that the corresponding hemicellulase plays a crucial role in the degradation of hemicellulose during the ensiling procedure.

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Table 1 The structural carbohydrates, monosaccharide and endogenous cellulolytic enzyme activity of alfalfa (Means $\pm$ SD).

Items [†]	Alfalfa
Cellulose (g/kg DM)	194 $\pm$ 8.01
Hemicellulose (g/kg DM)	114 $\pm$ 9.14
Glucose (g/kg DM)	4.42 $\pm$ 0.89
Mannose (g/kg DM)	0.078 $\pm$ 0.01
Galactose (g/kg DM)	0.716 $\pm$ 0.08
CMCase (U/kg FW)	81.4 $\pm$ 6.62
Avicelase (U/kg FW)	30.5 $\pm$ 4.03
β -glucosidase (U/kg FW)	55.7 $\pm$ 5.87
FPase (U/kg FW)	34.7 $\pm$ 8.39

[†] DM, dry matter; FW, fresh weight.

Table 2 The change of structural carbohydrates, monosaccharide and cellobiose during ensiling.

Values in the same row (a–c) with different superscript letters are significantly difference ($P < 0.05$).

Items	Ensiling days						SEM	<i>P</i> -value
	3	6	10	15	30	60		
Cellulose (g/kg DM)	177	178	180	179	180	185	0.177	0.720
Hemicellulose (g/kg DM)	99.2	98.2	97.5	93.2	89.6	87.3	0.224	0.240
Glucose (g/kg DM)	2.53 ^b	3.61 ^b	4.07 ^b	6.56 ^a	2.87 ^b	3.39 ^b	0.301	<0.001
Mannose (g/kg DM)	0.255 ^c	0.313 ^{abc}	0.391 ^a	0.377 ^{ab}	0.265 ^c	0.274 ^{bc}	0.023	0.004
Galactose (g/kg DM)	0.477 ^{ab}	0.484 ^{ab}	0.664 ^a	0.749 ^a	0.328 ^b	0.465 ^{ab}	0.038	0.011
Cellobiose (g/kg DM)	0.062 ^c	0.347 ^b	0.442 ^b	0.352 ^b	0.369 ^b	0.697 ^a	0.047	<0.001

Screening and Application of Coumarin Degrading Bacteria in Sweet Clover Silage

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Keywords: coumarin, degrading bacteria, sweet clover, silage

Introduction Sweet clover (*Melilotus officinalis* (L.) Pall.) is an annual or biennial herbaceous plant of the genus, which is notable for its abundant crude protein content. Coumarin, a secondary metabolite, is present in various plants such as sweet clover, Asiatic plantain (*Plantago asiatica* L.), and cherry (*Prunus pseudocerasus* Lindl.). However, when sweet clover is used for grazing, as fresh fodder, hay, or silage, there is a significant risk of livestock poisoning. This is because molds can transform coumarin into dicoumarol. Biodegradation, which can efficiently avoid the high cost and potential of secondary pollution, has already been employed in several studies. Hence, the principal objective of this study is to isolate a strain from sweet clover that exhibits high efficacy in degrading coumarin. By adopting this approach, we aim to diminish the transformation of dicoumarol and, in turn, avert livestock poisoning.

Materials and methods Isolation and degradation characteristics were performed in mineral medium (MSM) with coumarin. Its physiological and biochemical characteristics were measured according to Gu et al. (2023). Sweet clover silage was treated with distilled water (CON), *Lactiplantibacillus plantarum* (C71, 1×10^6 cfu/g FW), *Acetobacter tropicalis* (AT7, 1×10^6 cfu/g FW), *Heyndrickxia Oleronia* (L9, 1×10^6 cfu/g FW). The fermentation quality of sweet clover silage, the content of coumarin and degradation rate were determined. The data on fermentation quality and coumarin content were analyzed using two-way ANOVA, with inoculants, ensiling days, and the interaction between inoculants and ensiling days set as fixed effects (SPSS 26.0).

Results and discussion A coumarin-degrading bacteria, designated as L9, was isolated from sweet clover and identified as *H. Oleronia* through 16S rRNA sequencing analysis. Two functional lactic acid bacteria C71 and AT7, which possess the ability to degrade mycotoxins, were employed in the degradation of coumarin. Treatments using strains L9, C71, and AT7 alone led to a significant decrease in pH and a significant increase in lactic acid content ($P < 0.05$). Notably, the lowest pH values were observed in the treatments C71 and AT7. The addition of AT7 resulted in the highest acetic acid concentration, while the lowest acetic acid content was detected in the treatment with L9 (Table 1). Compared with L9 and the control, the inoculants C71 and AT7 significantly decreased protein degradation ($P < 0.05$). The addition of C71, AT7, and L9 caused different degrees of reduction in the coumarin content ($P < 0.05$), with the corresponding degradation rates being 72.38%, 68.11%, and 66.22%, respectively (Fig. 1). These results suggest that the L9 isolated from sweet clover is capable of effectively degrading coumarin, and the mycotoxin-degrading bacteria (C71 and AT7) can also degrade coumarin efficiently.

Conclusions *H. Oleronia* L9, isolated from sweet clover, has the capability to degrade coumarin. Moreover, two other strains C71 and AT7, are also highly efficient at degrading coumarin. To a considerable extent, the addition of C71, AT7, and L9 can significantly improve the fermentation quality.

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Table 1 Effect of coumarin degrading bacteria on the fermentation quality of sweet clover

Item	Treatment	Days of Ensiling			SEM	P-value		
		15	30	60		D	T	D×T
pH	CON	4.87 ^A	4.80 ^A	4.51 ^A	0.055	<0.001	<0.001	0.998
	C71	4.25 ^{Ba}	4.14 ^{Ca}	3.86 ^{Cb}				
	AT7	4.25 ^{Ba}	4.12 ^{Ca}	3.87 ^{Cb}				
	L9	4.49 ^{ABa}	4.44 ^{Ba}	4.19 ^{Bb}				
Lactic acid (g/kg DM)	CON	34.98 ^B	33.10 ^B	41.01 ^B	1.686	0.006	<0.001	0.916
	C71	60.91 ^A	55.05 ^A	61.10 ^A				
	AT7	53.55 ^A	52.50 ^A	57.71 ^A				
	L9	53.68 ^{Aa}	48.24 ^{Ab}	57.60 ^{Aa}				
Acetic acid (g/kg DM)	CON	6.47 ^{AB}	6.49 ^B	6.45 ^{AB}	0.338	0.896	<0.001	0.896
	C71	5.66 ^B	5.18 ^C	5.13 ^B				
	AT7	7.61 ^A	8.02 ^A	7.74 ^A				
	L9	2.46 ^C	2.60 ^D	2.55 ^C				
Lactic acid/Acetic acid	CON	5.37 ^C	5.09 ^D	6.36 ^C	1.045	0.001	<0.001	0.157
	C71	10.75 ^B	10.64 ^B	12.18 ^B				
	AT7	7.06 ^C	6.55 ^C	7.48 ^C				
	L9	21.85 ^{Aab}	18.52 ^{Ab}	22.68 ^{Aa}				
Propionic acid (g/kg DM)	CON	2.98 ^A	4.99	1.05	0.244	<0.001	<0.001	0.080
	C71	1.59 ^B	1.53	ND				
	AT7	1.38 ^B	1.57	ND				
	L9	1.75 ^B	2.11	1.41				

SEM, standard error of means; DM, dry matter; CON, control; C71, *Lactiplantibacillus plantarum*; AT7, *Acetobacter tropicalis*; L9, *Heyndrickxia Oleronia*. D, days of ensiling; T, treatments; D×T, interaction between days of ensiling and treatments. A-C Values with different superscripts differ significantly among days of ensiling on the same treatment; a-b Values with different superscripts differ significantly among treatments on the same ensiling day.

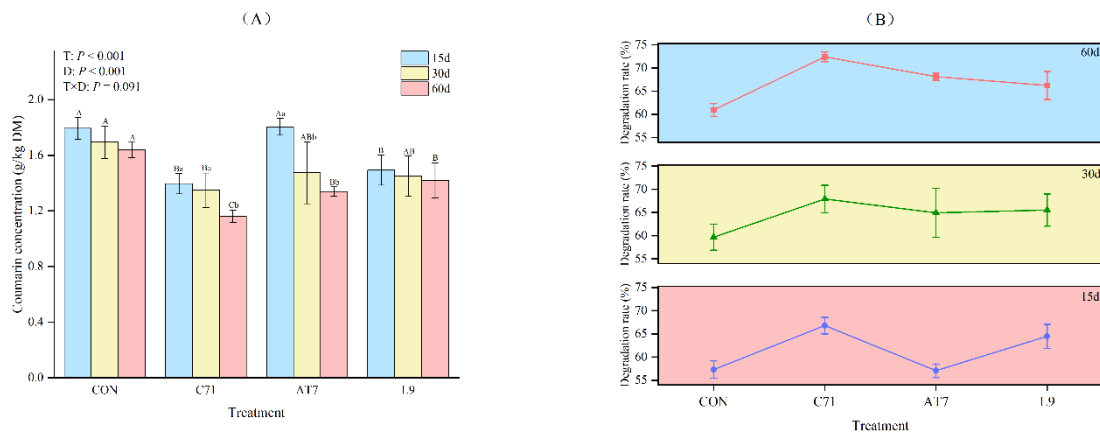


Fig. 1 Content and degradation rate of coumarin in ensiling. (A) Coumarin content in different ensiling days. (B) Coumarin degradation rate in different treatments.

***In vitro* Nutrient Fermentation and Methane Mitigation Potential of Pineapple By-product Silage in Ruminants**

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Keywords: greenhouse gas, propionic acid, degradation, tropical grass, fruit by-products

Introduction

Extreme conditions, such as high ambient air temperatures due to climate change, can negatively affect the nutritional quality of forages and crops in tropical regions, often reducing their concentrations of crude protein while increasing those of fiber (Daniel et al., 2019; Kyawt et al., 2020). Feeding high-fibrous materials to ruminants can increase enteric methane production, a potent greenhouse gas contributing to global warming (Jiyana et al., 2022). Reducing dietary concentrations of structural carbohydrates, which are primarily fermented to acetic acid by rumen microbes, and increasing the concentrations of non-structural carbohydrates, such as starch, promotes the ruminal production of propionic acid, thereby reducing enteric methane yield (Eckard et al., 2010; Hart et al., 2015; Shibata and Terada, 2010). Silages derived from agricultural and fruit by-products such as pineapple are practical alternatives in tropical ruminant feeding during the dry season (Kyawt et al., 2020). Properly fermented silages will produce favorable lactic acid content, which can be converted in the rumen to propionic acid (Kung et al., 2018), potentially lowering enteric methane. While pineapple by-products have been recognized as potential alternatives, the specific impact, particularly ensiled pineapple by-products, on methane production and rumen fermentation dynamics is not well quantified. Hence, this study aims to determine the *in vitro* gas production, methane yield, volatile fatty acids profile, and dry matter degradability of pineapple by-product silage after 48 hours of *in vitro* incubation and evaluate the effect of mixing pineapple by-product silage with tropical grass (*Urochloa brizantha* cultivar Toledo grass) on mitigating methane emission.

Materials and methods

The study was conducted at the International Center for Tropical Agriculture (CIAT, Colombia). Previously oven-dried and ground samples of *Urochloa brizantha* cultivar Toledo (CIAT 16835) were used. Additionally, samples of pineapple by-product silages, ensiled for 60 days, with and without the addition of two different bacterial inoculants, with four replicates per treatment were used. After the ensiling period, the silages were freeze-dried, milled, and pooled by treatment before *in vitro* incubation.

For the *in vitro* experiment, six treatments were established: (T1) pineapple by-product silage without inoculant, (T2) pineapple by-product silage inoculated with *Lentilactobacillus buchneri* (Magniva Steel, Lallemand), (T3) pineapple by-product silage inoculated with *L. buchneri* and homofermentative lactic acid bacteria (*Lactobacillus plantarum*, *Pediococcus acidilactici*, *Enterococcus faecium*, *Ligilactobacillus salivarius*) with enzymes (alpha-amylase, hemicellulase, xylanase, and cellulase; Magniva Classic, Lallemand), (T4) Toledo grass, (T5) 30% of T1 mixed with 70% of T4, and (T6) a mixture of 9% of T1 and 91% of T4 to obtain a balanced rumen nitrogen balance (GfE, 2001).

The dry matter of the pre-dried samples was determined by oven-drying at 105°C for 24 hours, followed by ashing at 550°C to determine crude ash (AOAC, 1990). The *in vitro* gas production test followed the protocol of Theodorou et al. (1994), using rumen fluid freshly collected from four fistulated Brahman cattle (450 ± 50 kg) that grazed *Cynodon nlemfuensis* with ad libitum access to water. The rumen fluid was blended and filtered through a 10-fold sterile hospital gauze. A total of 85 ml of the prepared mineral mix with buffer solution and 10 ml of filtered rumen fluid was added to glass bottles containing 1 g of sample. Samples of each treatment were incubated in a water bath at 39°C for 24 and 48 hours, with three replicates per treatment and incubation time. After incubation, total gas production was recorded, and two sub-samples (à 10 ml) of the accumulated gas were extracted and analyzed for methane using a gas chromatograph (Shimadzu GC-2014, Kyoto, Japan). Two sub-samples (à 10 ml) of the inoculum were centrifuged and analyzed for volatile fatty acids using high-performance liquid chromatography (Shimadzu 20A Series, Kyoto, Japan). Then, residual inoculum and sample material were filtered using a vacuum pump, and the solid residue was oven-dried at 105°C for 24 hours to estimate dry matter degradability.

Cumulative gas production and methane yield were analyzed using a linear mixed-effects model following a factorial design, with treatments and incubation time as independent factors. Volatile fatty acid concentrations and dry matter degradability were assessed using a generalized least-squares model. All analyses were performed using R[®] software version 4.4.0.

Results

Cumulative gas production (mL/g organic matter; $p < 0.01$) and methane yield (L/kg organic matter; $p < 0.05$) after 24 and 48 hours of incubation were greater for T1, T2, and T3 than for T4, T5, and T6, with T4 showing the lowest values.

Accordingly, T1, T2, and T3 had the highest concentrations of acetic acid and the lowest for T4 ($p < 0.01$), irrespective of the incubation time. However, the proportion of acetic acid to total acids was greatest for T4, T5, and T6, with T3 showing the lowest ($p < 0.01$). Propionic acid concentrations were greater for T1, T2, and T3 than for T4, T5, and T6 ($p < 0.01$), being lowest for T4 and T6. This was reflected in the higher proportion of propionic acid relative to total acids for T2 and T3 and the lowest for T5 and T6 ($p < 0.01$). On the other hand, Treatment 3 showed the highest butyric acid concentration, and Treatment 4 had the lowest after 24 and 48 hours of incubation ($p < 0.01$). The proportion of butyric acid relative to total acids was highest in T3 and T5 after 24 hours of incubation ($p < 0.01$), but after 48 hours, T1, T2, T3, and T5 exhibited the highest, and T4 was consistently the lowest ($p < 0.01$).

Dry matter degradability was greatest for T1, T2, and T3 and lowest for T4 after both 24 and 48 hours of incubation ($p < 0.01$).

Conclusions

In vitro gas production, methane yield, volatile fatty acid concentrations, and dry matter degradability were greater for silages from pineapple by-products compared to Toledo grass, indicating a more intensive and superior fermentation of nutrients within the silage. However, the fermentability of organic matter as a key indicator in determining the methane yield was not determined in this study. Combining the pineapple by-product silage with diets rich in starch to shift the fermentation towards propionic acid can be considered to mitigate methane. To further understand the implications of feeding pineapple by-product silage to ruminants, measuring the methane yield at the animal level should be explored to assess how the diet is digested and metabolized under natural conditions.

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Effect of Microbial Inoculants on Fermentation Quality of *Arachis pintoi* CV Florigraze Silages

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Keywords: additive, *Arachis*, organic acid, wilting

Introduction

Arachis pintoi is a legume from tropical climates with good adaptation in the subtropics, high crude protein and digestibility; ensiling may have the potential to support higher production levels in animals because of increased crude protein on diet. Most recently, *Arachis* ensilage associated with a combination of inoculants showed a positive linear effect on lactic acid concentration and a positive quadratic effect on lactic acid bacteria population (Gomes et al, 2021). Treatments with *Lactobacillus buchneri* (heterolactic bacterium) and more traditional homofermentative strains like *Lactobacillus plantarum* can result in a lower silage pH compared with an untreated legume silages (Kung Jr et al, 2018). In summary, the combination of techniques with the application of LAB inoculants could improve the fermentation parameters of *Arachis* silages, resulting in a higher quality feed for livestock. This research aimed to evaluate the fermentation profile of *Arachis pintoi* cv Florigraze with direct cut and wilted, with or without inoculants.

Material and Methods

The study was conducted from August to November 2021, at the Institute of Food and Agricultural Sciences, Marianna, Florida, USA. A factorial arrangement (2 x 4) in a completely randomized design was used, with four replicates. The *Arachis pintoi* cv Florigraze was harvested at 26% dry matter (direct-cut) and another material of *Arachis pintoi* was wilted for 6 hours until reach the dry matter of 54% (wilted). The grass was not mechanically chopped into small particles to mimic baleage, a common practice in the region. At ensiling, the first treatment was without inoculant (control) for each, direct cut and wilted forage. Then three types of commercial inoculants were applied per treatment with this label composition: 1. based on *Lactobacillus plantarum* (1×10^4 colony-forming units (cfu) g⁻¹ fresh forage), *Pediococcus pentosaceus* (9×10^4 cfu g⁻¹ fresh forage), and enzymes (β -glucanase, α -amylase, xylanase and β -mannanase), 2. based on *Propionibacterium freudenreichii* (2×10^4 cfu g⁻¹ fresh forage), *Pediococcus pentosaceus* (1×10^5 cfu g⁻¹ fresh forage), and enzymes (β -glucanase, xylanase and β -mannanase) 3. based on *Lactobacillus buchneri* (4×10^5 cfu g⁻¹ fresh forage), *Pediococcus pentosaceus* (1×10^5 cfu g⁻¹ fresh forage) and enzymes (β -glucanase, xylanase and β -mannanase) according to the manufacturer's recommendations. After treatments, the forage was ensiled into 5L mini silos at approximate density of 550 kg fresh forage m⁻³. The experimental silos were opened 60 days after ensiling. Dry matter was determined by drying at 105°C for 24 h (NFTA, 1993). Nitrogen was determined by combustion (Leco Instruments, Inc., St. Joseph, MI) (AOAC 976.06, 1990) and multiplied by 6.25 to obtain CP. Fermentation analysis of *Arachis pintoi* silage samples was performed by Dairy One (DHI Forage Testing Laboratory, Ithaca, NY). Samples were filtered through a disposable syringe filter [Millipore Millex 5.0 μ m PVDF (Durapore) membrane, Millipore Corp., Billerica, MA] and analyzed for acetic, propionic, butyric, and isobutyric acids using gas chromatography (Supelco, 1990; procedures cited from Lombard and Dowell, Jr., 1962). Lactic acid was determined using an YSI 2700 SELECT Biochemistry Analyzer equipped with an L-lactate membrane. Statistical analysis was performed with SAS 9.3 program. When there was significance by F test, means were compared by Tukey test at 1% probability.

Results

There were effects of interaction of wilting and inoculant ($P < 0.01$) for dry matter, lactic acid, acetic acid, lactate:acetate ratios, propionic acid, total acid, pH, crude protein, ammonia and ammo-N (Table 1). In our study the butyric acids were not detected. The better results of lactic acid were for wilted silages with inoculants compared with direct-cut silages. Wilted silages with *Lactobacillus buchneri* and *Pediococcus pentosaceus* were with the lower lactate:acetate ratio of all wilted silages with inoculants. Silages with very high lactic acid:acetic acid ratios may sometimes be more aerobically unstable than those with normal ratios (2.5-3.0) because low concentrations of acetic acid may not be sufficient to inhibit lactate assimilating yeasts. The control of wilted silages (without additive) had higher pH and the presence of inoculant reduced the pH. According to Kung Jr et al. (2018), with increases of DM above 40 to 45%, silage pH increases because metabolic water available for growth of lactic acid bacteria starts to become limiting as silage DM increases.

Table 1 Dry matter, fermentation products parameters, pH and crude protein of *Arachis pinto* silages

Item	Direct-cut				Wilted				SE ⁵	P-value ⁶		
	Wi ¹	LP+P ²	Pro+P ³	LB+P ⁴	Wi	LP+P	Pro+P	LB+P		W	I	W x I
Dry matter, %	27.02 ^c	26.26 ^c	26.85 ^c	26.38 ^c	51.51 ^b	51.46 ^b	60.17 ^a	58.46 ^{ba}	1.058	**	**	**
Lactic acid, %	5.88 ^a	5.21 ^b	4.99 ^b	3.08 ^c	0.67 ^e	2.50 ^c	1.39 ^d	1.27 ^{de}	0.212	**	**	**
Acetic acid, %	1.03 ^c	1.71 ^b	1.65 ^b	2.38 ^a	0.06 ^d	0.18 ^d	0.06 ^d	0.21 ^d	0.078	**	**	**
Lactic/Acetic Ratio	5.94 ^d	3.09 ^d	3.05 ^d	1.30 ^d	11.47 ^{bc}	14.74 ^b	22.25 ^a	6.42 ^{cd}	1.758	**	**	**
Propionic acid, %	0.00 ^c	1.39E-17 ^c	0.03 ^b	1.00 ^a	125E-18 ^c	937E-19 ^c	371E-19 ^c	2.69E-16 ^c	0.004	**	**	**
Total Acids, %	6.93 ^a	6.93 ^a	6.66 ^a	5.55 ^b	0.73 ^e	2.68 ^c	1.45 ^d	1.48 ^d	0.229	**	**	**
pH	4.33 ^e	4.58 ^d	4.55 ^d	4.78 ^c	5.63 ^a	4.43 ^{de}	5.10 ^b	4.83 ^c	0.061	**	**	**
Crude Protein, %	15.10 ^b	15.34 ^{ab}	14.41 ^c	15.41 ^a	14.41 ^c	14.08 ^d	14.27 ^{cd}	14.43 ^c	0.106	**	**	**
Ammonia, CPE ⁷ %	0.67 ^c	0.99 ^a	0.87 ^b	1.07 ^a	0.31 ^d	0.24 ^d	0.21 ^d	0.29 ^d	0.034	**	**	**
Amm-N, % of Total Nitrogen	4.46 ^c	6.48 ^{ab}	6.00 ^b	6.92 ^a	2.13 ^d	1.72 ^{de}	1.47 ^e	1.97 ^{de}	0.224	**	**	**

a,b,c,d,e Means within a row with different superscripts differ (Tukey's test, $\alpha = 0.01$).

¹ Wi: without inoculant; ² LP+P: *Lactobacillus plantarum* and *Pediococcus pentosaceus*; ³ Pro+P: *Propionibacterium freudenreichii* and *Pediococcus pentosaceus*; ⁴ LB+P: *Lactobacillus buchneri* and *Pediococcus pentosaceus*; ⁵ Standard error of the mean. ⁶ P-values for wilting effect (W), inoculant effect (I) and their interaction (W × I); ⁷ CPE: Crude Protein Equivalent;

Conclusions

The inoculants improve the fermentation process of *Arachis pinto* silages. These silages in general had a good fermentation profile, even considering the long particle size (no chopping) simulating baleage practice.

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Effect of Microbial Inoculants on Fermentation Quality of Limpograss Silage

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Keywords: additive, *Hemarthria altissima*, organic acid, wilting

Introduction

Limpograss is a warm-season perennial grass that maintains its digestibility even in later stages of maturity and tolerates poorly drained soils (Vendramini et al, 2010), which makes one satisfactory alternative for low-cost feeding. Grasses ensilage can be associated with a combination of inoculants that contain both *Lactobacillus buchneri* (heterolactic bacterium) and more traditional homofermentative strains like *Pediococcus pentosaceus* or *Lactobacillus plantarum* for the purpose to achieve an efficient fermentation and a rapid reduction in silage pH. This research aimed to evaluate the fermentation profile of limpograss with direct cut and wilted, with or without inoculants.

Material and Methods

The study was conducted from August to November 2021, at the Institute of Food and Agricultural Sciences, Marianna, Florida, USA. A factorial arrangement (2 x 4) in a completely randomized design was used, with four replicates. The limpograss *Hemarthria altissima* cv Gibtuck was harvested at 24% dry matter (direct-cut) and another material of limpograss was wilted for 6 hours until reach the dry matter of 40% (wilted). The grass was not mechanically chopped into small particles to mimic baleage, a common practice in the region. At ensiling, the first treatment was without inoculant (control) for each, direct cut and wilted forage. Then three types of commercial inoculants were applied per treatment with this label composition: 1. based on *Lactobacillus plantarum* (1×10^4 colony-forming units (cfu) g⁻¹ fresh forage), *Pediococcus pentosaceus* (9×10^4 cfu g⁻¹ fresh forage), and enzymes (β -glucanase, α -amylase, xylanase and β -mannanase), 2. based on *Propionibacterium freudenreichii* (2×10^4 cfu g⁻¹ fresh forage), *Pediococcus pentosaceus* (1×10^5 cfu g⁻¹ fresh forage), and enzymes (β -glucanase, xylanase and β -mannanase) 3. based on *Lactobacillus buchneri* NCIMB40788 (4×10^5 cfu g⁻¹ fresh forage), *Pediococcus pentosaceus* (1×10^5 cfu g⁻¹ fresh forage) and enzymes (β -glucanase, xylanase and β -mannanase) according to the manufacturer's recommendations, and the fourth treatment was without inoculant (control). After treatments, the forage was ensiled into 5L mini silos at approximate density of 550 kg fresh forage m⁻³. The experimental silos were opened 60 days after ensiling. Dry matter was determined by drying at 105°C for 24 h (NFTA, 1993). Nitrogen was determined by combustion (Leco Instruments, Inc., St. Joseph, MI) (AOAC 976.06, 1990) and multiplied by 6.25 to obtain CP. Fermentation analysis of limpograss silage samples was performed by Dairy One (DHI Forage Testing Laboratory, Ithaca, NY). Samples were filtered through a disposable syringe filter [Millipore Millex 5.0 μ m PVDF (Durapore) membrane, Millipore Corp., Billerica, MA] and analyzed for acetic, propionic, butyric, and isobutyric acids using gas chromatography (Supelco, 1990; procedures cited from Lombard and Dowell, Jr., 1962). Lactic acid was determined using an YSI 2700 SELECT Biochemistry Analyzer equipped with an L-lactate membrane. Statistical analysis was performed with SAS 9.3 program. When there was significance by F test, means were compared by Tukey test at 5% probability.

Results

There were interaction effects of wilting and inoculant ($P < 0.05$) for dry matter, lactic acid, acetic acid, lactate to acetate ratios, butyric acid, and VFA score (Table 1). After active fermentation, *L. buchneri* slowly converts some lactic acid to acetic acid (Borreani et al., 2018) and these results were observed for wilted silages with *Lactobacillus buchneri* and *Pediococcus pentosaceus*. In spite of differences in direct-cut and wilted silages, lactate to acetate ratios were high (~ 5.0) in all wilted silages, except for silages with *Lactobacillus buchneri* and *Pediococcus pentosaceus*. The control of wilted silages (without additive) had higher pH and the presence of inoculant reduced the pH. Dairy One Forage Lab developed a VFA Score. The score weighs the positive impact of lactic and acetic acids against the negative impact of butyric acid to arrive at a single value for evaluating silage quality. The concept is similar to Relative Feed Value, which is a score used to evaluate forage feeding value. The score will be between 0-10, with higher scores indicative of better fermentation: 8-10 (good), 6-8 (satisfactory), 3-6 (needs improvement) and <3 (poor). Wilted silages (except for silages with *Lactobacillus buchneri* and *Pediococcus pentosaceus*) and direct cut without inoculants were satisfactory.

Table 1 Dry matter, fermentation products parameters, pH and crude protein of limpgrass silages

Item	Direct-cut				Wilted				SE ⁵	P-value ⁶		
	Wi ¹	LP+P ²	Pro+P ³	LB+P ⁴	Wi	LP+P	Pro+P	LB+P		W	I	W x I
Dry matter, %	22.45 ^c	22.29 ^c	21.71 ^c	24.07 ^c	36.94 ^a	38.67 ^a	31.81 ^b	29.06 ^b	1.647	***	**	**
Lactic acid, %	5.92 ^a	3.12 ^b	2.73 ^{bc}	2.16 ^c	2.01 ^c	3.17 ^b	3.13 ^b	2.46 ^{bc}	0.104	**	**	***
Acetic acid, %	1.15 ^{ab}	1.02 ^c	0.79 ^b	0.97 ^{ab}	0.16 ^c	0.65 ^{bc}	0.72 ^b	1.46 ^a	0.038	ns	*	**
Lactic/Acetic Ratio	5.26 ^b	3.82 ^{bcd}	4.07 ^{bc}	2.30 ^{cd}	12.62 ^a	5.13 ^b	5.18 ^b	1.73 ^d	0.584	**	***	***
Propionic acid, %	0.02 ^{bc}	0.07 ^{bc}	0.17 ^b	0.09 ^{bc}	1.4E-16 ^c	1.75E-16 ^c	0.33 ^a	0.01 ^c	0.003	ns	**	ns
Butyric acid, %	0.06 ^c	2.15 ^b	3.29 ^a	3.04 ^{ab}	1.65E-15 ^c	8.53E-16 ^c	0.03 ^{ab}	0.06 ^c	0.094	***	***	***
Total Acids, %	7.16 ^a	6.37 ^{ab}	6.98 ^{ab}	6.27 ^b	2.17 ^d	3.82 ^c	4.19 ^c	3.98 ^c	0.103	***	**	*
pH	3.88 ^c	4.33 ^b	4.35 ^b	4.35 ^b	4.77 ^a	4.23 ^b	4.30 ^b	4.40 ^b	0.004	**	ns	***
Crude Protein, %	7.74 ^a	7.47 ^{ab}	6.87 ^{abc}	5.72 ^{cd}	4.77 ^d	4.68 ^d	4.91 ^d	6.02 ^{bcd}	0.358	**	ns	*
Ammonia, CPE ⁷ %	0.54 ^{bc}	0.70 ^a	0.69 ^a	0.59 ^{ab}	0.31 ^e	0.30 ^e	0.39 ^{de}	0.44 ^{cd}	0.002	***	*	*
Amm-N, % of Total Nitrogen	7.13 ^c	9.36 ^{abc}	10.17 ^{ab}	10.68 ^a	6.71 ^c	6.62 ^c	7.93 ^{bc}	7.48 ^c	0.960	**	ns	ns
VFA Score ⁸	8.48 ^a	5.15 ^{cd}	4.01 ^d	2.23 ^e	7.25 ^{ab}	7.27 ^{ab}	7.23 ^{ab}	6.00 ^{bc}	0.289	***	***	**

a,b,c,d,e Means within a row with different superscripts differ (Tukey's test, $\alpha = 0.05$).

¹ Wi: without inoculant; ² LP+P: *Lactobacillus plantarum* and *Pediococcus pentosaceus*; ³ Pro+P: *Propionibacterium freudenreichii* and *Pediococcus pentosaceus*; ⁴ LB+P: *Lactobacillus buchneri* and *Pediococcus pentosaceus*; ⁵ Standard error of the mean. ⁶ P-values for wilting effect (W), inoculant effect (I) and their interaction (W × I); ⁷ CPE: Crude Protein Equivalent; ⁸ VFA: Volatile fatty acids

Conclusions

The inoculants did not significantly improve the fermentation process. These silages in general had a good fermentation profile, even considering the long particle size (no chopping) simulating baleage practice.

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Detecting Aerobic Changes in Silages and Silage-based Rations on Farms and in Laboratory Scale Silos – Part B: Technical Setup

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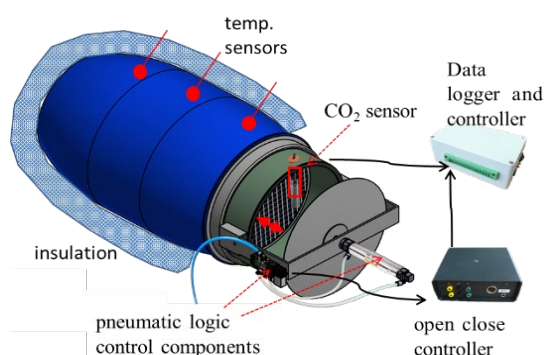
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Keywords: aerobic deterioration, Emissions, GHG, pH change, temperature change, O₂ consumption

Introduction

Aerobic changes cause high nutrient losses in silage making all over the world. Especially the silo face of good fermented high-energy silages tends to aerobic spoilage (Pitt et al., 1993). High summer temperatures general intensify the problem. Different factors like, chop length, compaction intensity, (DM), and feed-out management impact the real-time air exposure before feeding. The other factor is silage stability during air exposure before microorganisms start reheating. Rising temperature is a simple to measure and most used indicator at the silo face to detect aerobic deterioration. However, collecting data about air infiltration, rising temperatures, and resulting gas losses is challenging at farm-scale silos. Even the effect of silage additives can only be compared by filling similar silos in parallel using the same forage crop and harvest technology. Tests in small-scale silos and glasses (1.5 L) offer the opportunity for airtight conditions during fermentation. Tests of aerobic stability are carried out in insulated boxes, and airflow is injected into the sample.

Methods



To get a more detailed view of the metabolism of the microbials and the consumption of O₂, the test setup can be modified in different ways. To simulate a free silo face, 60L buckets are filled with silage crops of different treatments (additives) at variable densities. The headspace can be closed to observe the outflow of CO₂; CO₂ concentration rises in the measuring time (120 s) of a 30min. interval. Three temperature sensors in 15 cm steps behind the face measure the silage temperature over time and show how far the active microbial metabolism extends into the silage behind the surface. The test setup shows the effect of silage additives on fermentation products, microbial counts, and the effect of varying densities on the intensity of reheating at the face and in deeper layers of the silage.

Figure 5: Silage bucket with open-close head space, CO₂, and temperature sensors

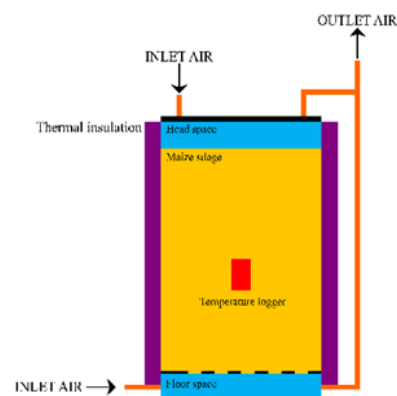


Figure 2: 30L bucket with head and floorspace for continuous gas flow

To capture all gas products during fermentation and aerobic storage, there are two different test setups possible, using gas bags during closed storage, and controlled airflow through the sample during the aerobic time. In the first setup, silage is compacted in 1.5 L glass jars, and gas bags are connected airtight. During the aerobic stability test, the silages are ventilated by an electronically controlled air flow (2L/h) directly into the sample by a 5mm steel pipe. This leads to aerobic conditions in the whole glass. The O₂ consumption caused by microbial activity is measured by the CO₂ concentration in the controlled outflow of the sample twice daily (gas chromatography). In this way, the DM losses and CO₂ sum can be calculated. The glasses stay in a box with thermal insulation and sensors for temperature recording.

(Figure 2) (Deeken et al., 2024b).

For a continuous measurement of the gases using a multi-gas monitor, a constant gas flow of higher volume has to be set up

Maize silage [CON (untreated), BIO (LAB hetero), CHE (sodium benzoate and potassium sorbate)] was fermented in buckets connected to gas bags (Deeken et al., 2024a). During aerobic storage, air was sucked through the head- and floorspace of the samples. This way, the silage is flooded with air from top and bottom. The multi-gas monitor takes gas from the outflow and analyses the concentration of CO₂ and ethanol.

Results

Collecting produced gases during the anaerobic storage and measuring gas concentration during the aerobic time at the surface and in ventilated samples showed differences in microbial activity in the silage and, thereby, the effect of silage additives. During anaerobic storage, the treated silage (heterofermentative LAB) exhibited 15-25% more gas in

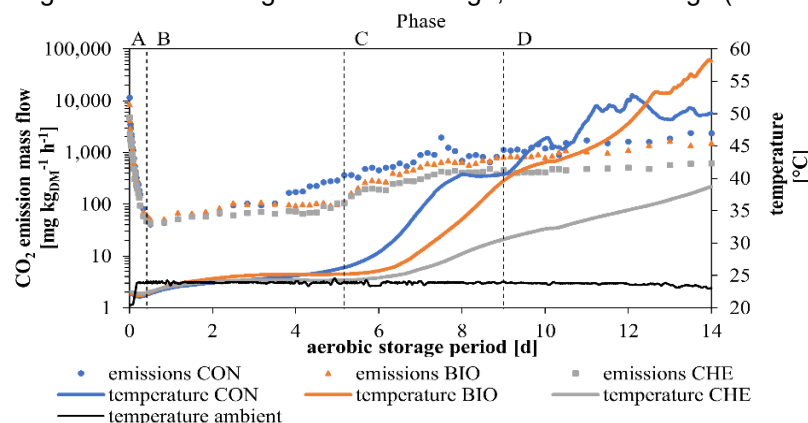


Figure 3: CO₂ emissions and silage temperatures during controlled air ventilation of various maize silage treatments.

deeper layers. To quantify gas production in anaerobic and aerobic time, closed storage combined with controlled ventilation and measuring the gases in the outflow are suitable methods. It shows the microbial activity in the sample and gives detailed information about gas production and silage losses. Differences caused by treatment with additives can be seen clearly. Reheated silages can consume 40 times more O₂ than stable silages during the aerobic phase. In a short time (2-3 days) of intensive reheating, silage emits more CO₂ than in a long time of closed storage before. For further aspects, please see Maack et al. (2025), Martens & Resch (2025), and Sun et al. (2025) in this volume.

the bags. However, during the aerobic phase, reheating of the untreated silages started quickly (after 2-4 days) and reached 200,000 ppm CO₂ in contrast to 8,000 ppm in the outflowing air in the case of cool silage in the LAB variant (Maack et al. (2025) this volume). Figure 3 shows the differences in the continuous measurement of CO₂ emissions and silage temperatures of the treatments CON, BIO, and CHE. Reheating starts after 4 days in CON, 5.5 days in BIO, and 6 days in CHE.

Conclusions

Measurements at the face of a high-volume silage sample (60L) can simulate a silo face and show the effects of oxygen penetration in

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Effect of a Silage Inoculant Containing *L. diolivorans* on CO₂ Emissions of Corn Silage During Anaerobic and Aerobic Storage

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Keywords: emission, carbon dioxide, aerobic stability, silage inoculant, *L. diolivorans*

Introduction

Emission measurement and gas absorption from silage is part of current research all over the world (Schmidt and Vigne, 2023; Deeken et al., 2024). Silage fed to cattle has a climate impact due to emissions (CO₂, nitrous gases, volatile organic compounds (VOC)) and DM losses that occur during anaerobic (Krommweh et al., 2021) and aerobic (Shan et al., 2021) storage conditions. According to Deeken et al. (2024), GHG and VOC emissions during storage, and especially under aerobic conditions during reheating, can be reduced by using silage additives. Ensuring aerobic stability of silages is therefore not only important for economic livestock and milk production, but also for the environment. Unfortunately, aerobic stability of silages can be insufficient if they have to be opened a few days after harvest. The use of appropriate silage inoculants based on heterofermentative lactic acid bacteria (LAB), such as *L. diolivorans*, has increased in recent years due to their effect on aerobic stability after a short storage period (Thaysen and Kramer, 2018). Therefore, the aim of this study was to evaluate the effect of a LAB mixture containing *L. diolivorans* on fermentation, aerobic stability and GHG emission of corn silage ensiled for 14 and 90 days.

Materials and methods

Chopped corn (41% DM) was ensiled in 1.5L mini silos (MS) and in 1.5L mini silos with integrated injection lance (MSIL) for 14 (OD14) and 90 days (OD90) at 20°C storage temperature in three replications.

Treatments consisted of an untreated control (CON) and treated (INO) with a mixture of homo - and heterofermentative LAB (*Bonsilage Speed M*: *L. diolivorans*, *L. buchneri* and *L. rhamnosus*; application rate of 250.000 CFU/ g FM). Air stress was simulated during storage after 7 days for a total of 24 hours. Forage samples of MS were analysed to determine forage quality (nutrients, pH-value, organic acids and alcohols). For MS, aerobic stability was measured according to Honig (1990). For MSIL, aerobic stability test was simulated by injection of 2L air per hour (Fig. 1) and exhaustion of air two times per day. In addition, CO₂ emissions were monitored for MSIL during anaerobic storage and during aerobic stability test. For statistical evaluation, the data were examined by SAS including Kruskal-Wallis test for significant differences ($P < 0.05$) between CON and INO.

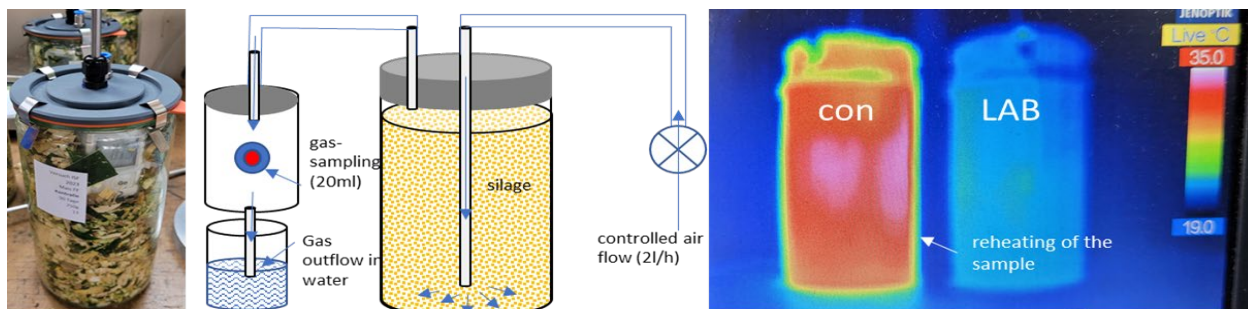


Figure 1. Left: experimental set up for aerobic stability and gas sampling using controlled air flow through the silage; right: thermogram of two mini silos (control vs. treatment) after 7 days of aerobic test

Results

For MS, lactic acid and acetic acid were increased and pH was significantly reduced in INO compared to CON at both ODs. INO moderately enhanced weight loss during anaerobic storage at both ODs, which may be related to the heterofermentative activity of *L. buchneri* and *L. diolivorans*. Furthermore, aerobic stability was improved at OD14 (CON: 1.3 d; INO: 8.0 d), while both CON and INO remained stable at OD90 (Tab. 1). In addition, CO₂ emissions during the anaerobic storage (OD14 and 90) were 2.9g and 4.5g for CON and 3.5g and 6.0g for INO (+20 % vs. CON). The aerobic stability of the corn silage ensiled in the MSIL differed from that of the MS at OD14 as a result of the air injection (Fig. 2, left). While the CON started to heat up after about one day, the INO remained stable during the 14 d of aerobic exposure. CO₂ emissions followed a similar pattern to the temperature pattern.

Table 1: Silage parameters of the corn silages after opening day 14 (OD14) and 90 (OD 90) for control (a) and treated LAB mixture (b) ensiled in mini silos (MS).

Parameter	OD14		OD90	
	a	b	a	b
DM _c (%)	44.4	43.6	43.3	42.5
LA (% DM)	1.74 ^a	2.58 ^b	2.13 ^a	2.45 ^b
AA (% DM)	0.59 ^a	1.67 ^b	0.97 ^a	3.13 ^b
pH	4.38 ^a	3.97 ^b	4.18 ^a	3.97 ^b
WL (% DM)	1.77	2.33	2.83	3.67
AS (days)	1.30 ^a	8.00 ^b	>14	>14

(DM_c = DM corrected, LA=Lactic acid; AA= Acetic acid; AS= Aerobic stability according to Honig, 1990 (max. length: 14 days); WL= Weight loss;

The observed mean CO₂ concentrations were significantly higher in CON (14.6%) in contrast to INO (0.76%), Fig. 2, right. At OD90, the aerobic stability and CO₂ emissions measured during the aerobic stability test were very low (CON: 0.76% and INO:0.72%) and did not differ between treatments, as both treatments showed no reheating during the 14 days of aerobic exposure.

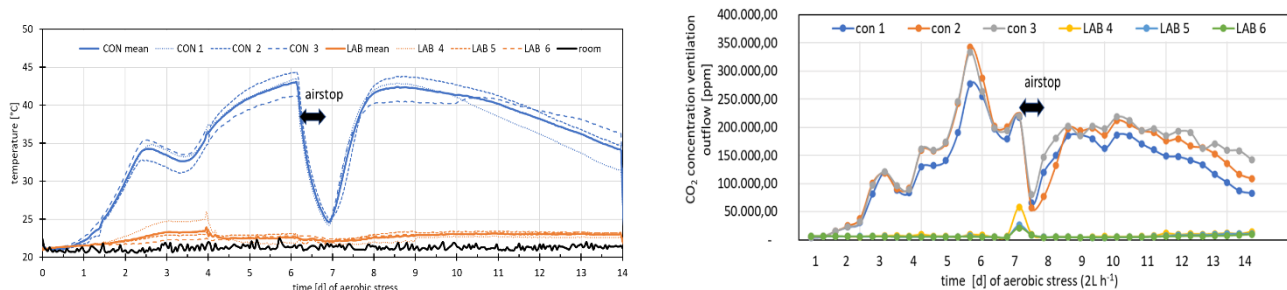


Figure 2: Silage temperature (left) and emitted CO₂ concentrations in the exhaust air (right) during aerobic stability test at opening day (OD) 14 for control (con1-3) and INO treated variants (LAB4-6) ensiled in the mini silos with injection (MSIL). Note: The air injection failed at day 7 for 14h, so the temperature and CO₂ concentrations dropped immediately.

The differences obtained in CO₂ concentration in the exhaust air during aerobic stability test at OD14 were due to intensive reheating and the associated respiration losses. Calculating the mass of emitted CO₂ based on the sum of air exhausted from the MSIL and the mean CO₂ concentration during 14 days of reheating, 192g CO₂ were emitted from 0.75kg FM corn silage ensiled in each MSIL, whereas only 9.7g CO₂ were emitted from one MSIL of the LAB treatment. The ventilation of the silage used in this study was a very intensive air stress and lead to high losses in CON with its low aerobic stability. This is not realistic for a whole silo in practice. However, it shows the potential for losses of instable corn silages. Therefore, it is strongly recommended to avoid losses during fermentation and especially during reheating to ensure economic livestock and milk production in order to minimize unnecessary emissions. Further tests in farm scale of short fermentation periods can help to investigate the effects in practice.

Conclusions

The present study demonstrates the effect of a LAB mixture containing *L. diolivorans* on aerobic stability and CO₂ emission losses during reheating process of corn silage after a short storage period. Thus, appropriate LAB mixtures have the potential to be part of emission reduction strategies in livestock production.

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Silage Troubleshooting - Part I: Silo Control Measurement of Physical Aspects

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Keywords: crop compaction, chop length, particle size, pH, temperature distribution, oxygen penetration, reheating

Introduction

During feedout, all silages are exposed to air at the silo face. The aerobic activity of yeasts and the subsequent heating of the silage depends on the fermentation products and the physical properties of the silage (Wilkinson et al., 2012). To get a quick overview of the risk of reheating and the prediction of losses at the silo face, some relevant parameters can be measured or observed.

Methods

Indicators to observe at the silo are leakages in the covering, silage temperature, pH, bulk density, and color as physical parameters. Additional sensory indicators (see Part II, Resch et al., this volume), the chemical composition of fermentation products (see Part IV, Schneider et al., this volume), and colony counts of microbial groups (see Part V, Wagner et al., this volume) give helpful information for a good assessment.

Measuring density often shows a relation between areas of low density and higher temperature, especially in the top layer of the silo face. High-energy silages with > 35% dry matter (DM) often suffer from reheating due to increased clamp pore volume. Silage stability during direct air contact is very variable and depends on the content of different fermentation products and the microbial counts at the opening (see also Part III, Martens et al., this volume). A well-done covering and bulk density > 230 kg DM m⁻³ at 30% DM and 270 kg DM m⁻³ at 38% DM, in combination with feedout rates of > 2 m per week, limit the air influx into the silage. Slow oxygen influx limits the microbial activity at the silo face.

Compaction of the crop in all parts of the silo during filling and complete covering with high-quality films are necessary to achieve a stable silo face. Small particles, as a result of short chopping, are easier to compact, resulting in higher density. Overlapping films from the silo wall to the top covering and tight placement of gravel bags are necessary to avoid air infiltration. During feedout, the parameters temperature, pH, and bulk density can be measured across different positions at the silo face using quite simple equipment. Density at the silo face can be measured by sampling of cores with a constant volume. In practice, the measurement error is quite high, mainly if small core diameters are used, and realizing many sampling points is time-consuming. Measuring penetration resistance using a standardized apex is an alternative method to estimate crop density. Electric-driven penetrometers with force mapping along the penetration way provide good results but need costly equipment (Li et al., 2016). Hand-used penetrometers are simple to use and offer to measure at different points of the face in a short time.

Hand penetrometer: The push force to use the penetrometer is generated manually. In highly compacted crops, up to 400 N is necessary. After the first function tests, a study was performed to calibrate the penetrometer to different material properties of maize silage (Maack et al., 2018).

Figure 1: Hand-pushed penetrometer with changeable spring and standard cone

To measure the temperature at the silo face, rod thermometers and thermo cams can be used. To identify reheating, measuring points 10- 50 cm in the crop are used because of the cooling effects directly on the surface. Thermograms of the silo face show differences in the whole surface and have to be taken in the early morning to avoid the effects of sun radiation. To look behind the surface, the picture can be taken directly after feedout by a silage cutter. Parts with high temperatures > 30°C predominantly suffer from longer air influx into the clamp.

Reheating of silage always leads to energy losses. Calculating the amount is quite difficult and depends on the intensity and time of reheating. New tests in small-scale silos detect the complete CO₂ flux during fermentation and time of reheating (Deeken et al., 2024). Energy losses due to reheating can exceed 10-15%, much more than the preceding fermentation losses.

In silage tests under controlled conditions, the effect of different bulk density or silage additives can be visualized using thermograms. When the insulation gets removed shortly before taking the thermogram, it is possible to measure almost the center temperature of small-scale silos. Even the activity of heterofermentative bacteria is indicated by higher temperatures in the closed buckets in the later fermentation period after 60 days of storage.

Figure 3: Silo face of maize without covering / 1.5L glasses with and without additive after aerobic stability test / 60L buckets (maize) of different density during open storage.

The pH of the silage is a simple indicator of the success of the ensiling process in grass and alfalfa. Values > 4.8 to 5.0 show that the silage may not be fermented well (Part IV, Schneider et al.).

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Silage Troubleshooting – Part III: Detecting Aerobic Changes on Farm

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Keywords: aerobic stability, aerobic deterioration, spoilage, indicators

Introduction

Aerobic instability has become a major problem in silage management (Borreani et al., 2018). How is it defined? Aerobic instability means any metabolism when silage is exposed to the air, which results in losses in dry matter, energy, and a decline in hygienic and nutritional quality. It refers to processes during the feed-out phase when the silo face is open or when the silo cover is damaged during ensiling, which leads to an exchange of gases and renewed oxygen supply inside the silage. When animals refuse to feed and milk production decreases (Gerlach et al., 2014; Borreani et al., 2018), the factors must be checked and controlled as an important aspect of silage assessment (Martens et al., 2025). This work suggests how to evaluate aerobic stability on farm.

Indicators

How can the potentially detrimental activities be detected easily by indicators on the farm, and how can they be interpreted? (For advanced methods, see Martens & Maack, in this volume.)

- Sensory evaluation is an important tool in the daily working routine of the staff handling the feed, may it be the driver of the feed mixer wagon or any other staff member who handles silage. Visual signs for reheating are steam at the silo surface, and spoilt parts are either dark or show cream-colored yeast points or mold mycelium. Olfactory, the odor of fruit esters or acetone may be perceived as a sign of esterification of alcohol and acid or of ammonia or rotting fish as a sign of proteic decomposition. When touched, the silage feels warm or has a smeary consistency (indicators; see also Part II, Resch et al., in this volume).

- Temperature increase indicates respiration heat due to microbial activity. Under farm conditions, the ambient temperature at the time of ensiling and, ideally, also the core temperature of the silo before sealing should be documented. It is recommended to measure the temperature at the silo face in the feed-out phase at different depths (10, 40, and 100 cm are proposed, Richter et al., 2009) as well as the ambient temperature (see also Part I, Maack et al., this volume). Borreani & Tabacco (2010) suggest 20 cm for maize silage bunkers as the first centimeters have usually adapted to the ambient temperature. In smaller silos, the core temperature should have stabilized at around 15 °C in temperate regions. Large bunker silos, however, have a heat-insulating effect. Thus, the inner core maintains the ensiling temperature for a long time. Considering all these factors, the temperature at a depth of 10 cm, which is > 5 K compared to the inner core, indicates reheating (DLG, 2025).

- pH value is another indicator, which is simple to determine using suitable indicator paper or handheld meters (see Part IV, Schneider et al., this volume). It is a relatively solid parameter within the silo face (Cherney & Cherney, 2003). As changes in the pH value are more indicative of lactic acid degradation than a single measurement, regular recording is desirable. The detection of a ΔpH of 0.25 is a clear sign of aerobic decomposition in maize silages (Gervasio et al., 2018). The pH tends to change slowly in grass and legume silages despite decreasing acid contents due to their higher buffering capacity (Martens, 2006). Reference pH values for some of the typical silages of the temperate zone before lactate degradation are listed here: maize: < 4.0; grass: 4.0-4.7 (from 20-45 % DM, depending on DM; DLG, 2004); lucerne: 4.2-4.9 (from 20-45 % DM, depending on DM).

Additional factors to check are the feedout rate and the feed intake.

Measures

Once aerobic spoilage is detected, the following immediate measures should be taken:

- Seal any unintended openings of the silo
- Discard visibly molded and rotten parts sparsely
- Accelerate the feed rate
- Freshly prepare the mixed ration before feeding, ideally several times a day
- Possibly apply stabilizers when mixing the ration (Martens *et al.*, 2022) after assessing the hygienic status (see Part IV, Wagner et al., this volume)

Analyze the causes of aerobic spoilage in the present silage to prevent future occurrence in the coming season:

- Measure silage density/porosity and compare to target values (see Part I, Maack et al., this volume)
- Recap on the circumstances of ensiling, especially the delay of covering (Weiss et al., 2022)

Drawing the necessary consequences will help to realize good agricultural practice in ensiling.

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Seeding Rate Effects on Morphology and Biomass Production of Sorghum x Sudan in Different Planting Seasons

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Keywords: Sorghum x Sudan, seeding rate, morphological responses

Introduction

Sorghums are resilient forage crops, often used in regions of high temperature and low rainfall (Millner et al., 2011) or when grown under stressful or resource limited conditions (Kaplan et al., 2019). Sorghum is often used as an alternative to corn for preserved forage because of the lower cost of production and versatility, as it can be bagged (silage), baled (baleage) or grazed. Sorghum x Sudan hybrids (*Sorghum bicolor* L. × *Sorghum sudanense*) are more commonly used in Southeast U.S. compared to forage sorghum (Beyaert and Roy, 2005; Millner et al., 2011; Ferraretto et al., 2018), because of its better regrowth capacity, better disease tolerance, and fewer seedheads (a desirable trait when used for grazing or baleage). While extensive research has focused on corn silage crop technologies and agronomic practices (Ferraretto et al., 2018), studies on sorghum x Sudan crop management, such as target planting date and plant population, remain limited. Therefore, this study aims to evaluate biomass production and morphological responses of sorghum x Sudan to varying seeding rates during two planting seasons.

Materials and methods

The study was conducted at the University of Florida Plant Science Research and Education Unit in Citra, Florida, during two growing seasons (spring and summer) in 2024. One sorghum x Sudan hybrid, 'Advanta AS6501', was planted on 17 April (spring planting) and 11 July (summer), at six seeding rates, in a randomized complete block design with four replicates. Treatments were 247,000; 370,500; 494,000; 617,500; 741,000; and 988,000 seeds ha⁻¹. Experimental units (1.5 x 6.1 m) were established using a Great Plains no-till drill adapted with a cone planter, in 19-cm center. Crop management followed UF/IFAS recommendations for forage sorghum (Vendramini et al., 2022), with fertilization rates of 170 kg N ha⁻¹, 56 kg P ha⁻¹ and 90 kg K ha⁻¹. These nutrients were applied in a pre-incorporated application, a stater dose and two additional applications during the growing season. Pest management included the application of Prowl (pendimethalin) and Dual (S-metolachlor) at planting, atrazine at approximately 30 cm of plant height, and a single insecticide application of Intrepid (methoxyfenozide). Irrigation was provided as needed throughout the trial. Plots were harvested with Wintersteiger silage plot harvester to a 10-cm stubble, twice during the growing seasons, once plants reached ~114 cm. Harvest dates were 7 June (51 days after planting) and 19 July (42 days after first cut) for spring; and 27 August (47 days after planting) and 3 October (37 days after first cut) for summer. Responses included number of seedlings four weeks after planting (prior to tillering), number of tillers and stalk diameter at 10 cm prior to each harvest. Once harvested a fresh subsample of the total biomass was weighed and then dried at 50°C for 96 hours to determine dry matter (DM) content and yield. Samples were analyzed for nutritive value using Near Infrared Reflectance spectroscopy. We first tested the relationship between seeding rate and realized seedlings, and then biomass yield and morphological responses to realized seedlings testing different linear and non-linear regression models. Best models were selected based on the corrected AIC and root mean square error.

Results

Overall, the realized number of seedlings was 10% greater in spring than summer. During spring, the number of seedlings increased linearly with the seeding rate up to 823,000 seeds ha⁻¹. In contrast, the realized number of seedlings increased linearly, without evidence of plateau, in the summer planting. Tiller number (tiller m⁻²) for both seasons and harvests increased linearly with seedlings density, mostly driven by a greater number of plants per unit area and less likely by an increase in tiller per plant. Conversely, the stalk diameter showed a negative linear response to seedling density, going from 94 mm to 71 mm, and was 43% smaller in the second harvest compared to the first. However, there was a compensation between the number of tillers and stalk diameter, where increases in number of tillers was associated with decreases in stalk diameter.

The first harvest in the spring showed a positive linear response to the number of realized plants and yield. In contrast, the second harvest showed an asymptotic yield response until 370,000 plants ha⁻¹, followed by a linear decline in yield above this breakpoint. Total biomass (i.e., the sum of the first and second harvests) linearly increased with the realized seedling numbers up to 469,000 plants ha⁻¹ (Figure 1). At this level, the predicted total biomass was 6,950 kg DM ha⁻¹, while above this level, there was no response in total biomass yield to further increases in seedling density. For the summer planting, both harvests exhibited a positive linear response, without an evidence of yield plateau in response to realized seedling (Figure 1). According to this model and the maximum realized seedlings of 741,000 plants ha⁻¹, the predicted total biomass yield reached 7,960 kg DM ha⁻¹. There was no significant seeding rate effect in any of the nutritive value parameters tested.

Figure 1. Total biomass yield (harvest 1 + harvest 2) in response to the number of realized plants, 4 weeks after planting, during spring and summer. Black lines indicate the best model in each case, and the blue lines indicate simulations based on bootstrap technique.

Conclusions

The seeding rate significantly affected the seedling density during spring and summer and morphological responses of sorghum x Sudan during both planting seasons. Increases in stalk diameter effectively compensated the lower tiller density in spring, suggesting that moderate seeding rates are enough to achieve potential yields. However, increases in stalk diameter were not enough to compensate the lower plant density in response to moderate seeding rates during summer. Different from expected, nutritive value was not affected by seeding rate treatments. This evidence indicates that higher seeding rates are needed in summer than in spring to achieve similar sorghum x Sudan yield potential, likely associated to higher stress conditions (heat) in the summer.

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Determining the Optimal Harvest Time to Corn Crops Using an Unmanned Drone with a Multispectral Camera

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Keywords: UAV (unmanned aerial vehicle), photogrammetry, nutritional analysis, optimal harvest time, spectrometry, corn

Introduction

Determining the optimal harvest time is crucial for maximizing maize silage quality and efficient cattle feeding. Recent extreme climatic variability caused fluctuations in biomass yield and quality traits like starch content and fiber digestibility. Accurate dry matter (DM) estimation is essential for harvest management. Traditional DM determination relies on labor-intensive sampling and lab analysis, limited in spatial scope. UAV-based multispectral sensing and forage harvester-mounted near-infrared (NIR) sensors offer rapid, non-invasive alternatives with better spatial coverage. This study compares DM estimates from UAV imagery, NIR sensors, and laboratory analyses at two Czech sites.

Materials and methods

In 2023, two sites (Nová Včelnice and Vanovice) were selected to compare dry matter (DM) estimation methods. Data acquisition was performed sequentially and in order: UAV imaging first, followed by laboratory sampling, and finally NIR measurements during harvest, within a four-hour window to minimize temporal variability and ensure comparability. Multispectral images were captured with a DJI Mavic 3M UAV, from which orthomosaics [3] and vegetation indices (NDVI, GNDVI) were derived. Regression models were applied to estimate DM content [1,2]. Reference samples for laboratory analysis were collected from precisely defined plots overlapping UAV coverage and harvester paths. During harvest, NIR measurements were obtained using the AgriNIR device at Nová Včelnice and the HarvestLab sensor at Vanovice. Key vegetation metrics derived from UAV data are summarized in Table 1.

Results

Only NIR sensors (AgriNIR, HarvestLab) scan whole matter from a field if we assume the defined accuracy of the NIR sensors than we can say:

- Nová Včelnice: the corn crop has 40.4 % DM with an error of 3 percentage points.
- Vanovice: the corn crop has 33.0 % DM with an error of 3 percentage points.

our lab. analyses and UAV confirm these statements. See tables 1 and 2.

Table 1. The DM content analysis of the corn field was measured by UAV. The summarization of average DM of the entire field (AF), average DM of the green part of the field (AGP), proportion of vegetation with optimal DM of the total area of vegetation (OPT), proportion of green vegetation (PGV).

Location	Actual state					Prediction (3 to 6 days) *			
	AF	AGP	OPT	PGV	NIR sensor	AF	AGP	OPT	PGV
	g kg ⁻¹	g kg ⁻¹	ratio	ratio	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	ratio	ratio
Nová Včelnice	430	420	12.0	96.0	404.3	475	463	6.00	90.10
Vanovice	347	347	76.9	100.0	330.0	385	385	56.5	100.0

* The prediction represents the field when we assume a constant increase in DM of 3 percentage points over the entire area of the scanned surface (the tabulated increase in dry matter is 0.5 to 1 percentage point per day)

We set the optimal dry matter (DM) content interval (OPT) between 300 and 400 g kg⁻¹, reflecting a medium-risk silage strategy based on our regression accuracy and agronomic practice to maximize starch energy. Table 2 shows the challenge of comparing DM content in chopped forage. The UAV measures heterogeneous vegetation, while the harvester-mounted NIR sensor measures homogeneous material, resulting in a narrower range for NIR. NIR sensors also detect fewer dry (low-yield) areas. Therefore, we recommend direct plant analysis when using UAV data. The UAV recorded low or zero-yield areas that artificially raised the average DM content (AF). Excluding these areas lowered the adjusted value (AGP), bringing it closer to NIR readings. AF and AGP are identical in uniform fields like Vanovice. PGV describes the canopy integrity of the field.

Table 2. The comparison of the dry matter content measured by different methodologies. NDVI, GNDVI and NIR sensors show an average value of a defined area.

Sample	Location A				Location B			
	NDVI	GNDVI	Lab. analysis	AgriNIR	NDVI	GNDVI	Lab. analysis	HarvestLab
	----- g kg ⁻¹ OM -----				----- g kg ⁻¹ OM -----			
Cut 1	436	425	382.7	380.9	374	411	318.6	326.8
Cut 2	442	437	371.2	405.6	360	407	324.1	329.3
Cut 3	563	459	354.8	391.5	345	404	318.1	327.8
Cut 4	399	397	405.8	407.5	347	399	323.6	326.5
Cut 5	430	433	379.7	393.8	337	384	318.1	328.8
Cut 6	457	433	406.2	380.9	340	379	319.0	340.8
3x5 plants	381	463	445.6	400.0	NA	NA	NA	NA
Field	451	431	NA	404.3	350	397	320.2	330.0

Conclusions

Drone measurements with a multispectral camera on heterogeneous vegetation yield results comparable or superior to conventional NIR sensors without extra calibration. This non-invasive method avoids plant damage. Algorithms reduce the impact of solar radiation, ensuring stability in varying weather. UAVs help track crop maturity over time and monitor dry matter content to determine the optimal maize silage harvest date (30–35%). Vegetation development can be predicted 3–6 days ahead. While less precise than lab analysis, it provides valuable data for harvest planning.

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A Century of Silage Quality Indices and Current Challenges

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Keywords: fermentation quality, sensory evaluation, Flieg key, DLG key

Introduction

As soon as silage gained importance in the seasonal feeding of farm animals, questions about its "quality" arose. This article shows the development of silage evaluation in the last century, focusing on the German-speaking area of Europe. It points out the current challenges and offers approaches.

History

The silage evaluation aim was: 1. Determining the fermentation quality to explain the conservation process and to get hints on palatability, digestibility, and feed value; 2. Determining the nutrient content and composition should ideally be evaluated in the farm animal to get the true feed value (Zimmer, 1957). Silage evaluation schemes were developed as early as the late 1920s in the German-speaking area: The animal breeder Werner Kirsch from the University of Königsberg probably invented a scoring system (Zimmer, 1959). He rated fermented feeds according to smell, taste, structure, and color, distributed points, and resulted in a final mark between "excellent" and "poor" (5 gradations). Oskar Flieg from the Agricultural Experimental Station Limburgerhof (belonging to the I.G. Farbenindustrie AG, the later BASF) included the lactic acid content and the ratio of the three fermentation acids (lactic, acetic, and butyric) to each other in a key which he published in 1938 (Flieg, 1938). They were based on the methodological developments by W. Lepper from the State Agricultural Research Institute Augustenberg (Lepper, 1938). At that time, formic acid was the predominant silage additive, which affected the analysis of lactic acid and butyric acid with the methods available at that time (Flieg, 1940). The so called Königsberg key, supplemented by the three analytical parameters pH, total acetic, and butyric acid, was included in the Book of Methods of the Association of German Agricultural Analytic and Research Institutes in 1951 (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA), 1951). In 1957, Ernst Zimmer from the Agricultural Research Centre in Braunschweig-Völkenrode published findings in which he compared results from sensory evaluation according to the existing schemes with the results from the chemical analysis of pH, lactic, acetic, and butyric acid and their rating according to Flieg (Zimmer, 1957). The accordance between sensory and chemical evaluation was > 80 % for sugar beet leaf, maize, and grass/grass-clover silages. A wrong assessment of the chemical analyses was given, especially if the grass silage was rotten or molded or if the structure of the sugar beet leaves was affected, and missing fermentation could not be detected by judging the ratio between the acids only. A wrong assessment of the sensory evaluation resulted from the weighting of structure and color, which in some cases outweighed the smell at that time. Thus, both approaches were seen as complementary. A new key for sensory evaluation was presented in 1959, called DLG-key for the evaluation of silage (Zimmer, 1959), including smell (now highest score), structure and color (least weighing), resulting in one of 4 marks from "very good" to "spoiled". The presence of molds and dirt was then included in the evaluation of the structure. Mold obviously became more important with the increasing proportion of wilted grass silages. In 1966, as a result of a meeting of the expert group for feed analysis of the VDLUFA, a new version of the Flieg key, later known as the Flieg-Zimmer key, was published with a revised calculation and scoring of lactic, acetic, and butyric acid contents and considering the sum of total acids (Zimmer, 1966). The butyric acid content was judged more strictly with the highest weighting, while no acetic acid and a high lactic acid content were desirable. In the wet silages, butyric acid was prevalent more frequently. Schmidt et al. (1972) took up the sensory evaluation complemented by evaluating the pH value dependent on the dry matter content. For the chemical evaluation, they used the revised Flieg score. They extended the NH₃-N proportion of the crude protein N content to indicate protein degradation (Schmidt et al., 1972). It became part of the state-run examination of feedstuff and was later disseminated as Lettin key in the German Democratic Republic (Schmidt & Beyrich, 1983), which was modified in 1987 (Weissbach, 1988). After the German reunification, a uniform DLG key for sensory and evaluation based on chemical analyses was adopted (Weissbach & Honig, 1992; DLG, 1997). It considered the fermentation qualities, which had changed because of the increasing use of biological silage additives. In 2004/2006, new adaptations were implemented (DLG, 2006; DLG, 2004). Because of environmental programs in Europe, the grasslands were used less intensively, which meant less fertilization, among others. The higher clostridial activity was rising because of very low NO₃ contents (Kaiser & Weiss, 2007; Kaiser et al., 1999). The DLG key then focused on dry matter and energy losses. That is why estimating energy content depending on the grassland's composition and its vegetative stage was included as a starting point to deduct points (and energy) for poor ensiling quality. A different sensory key for maize silage was developed for the first time. In Austria and Switzerland, these keys were adapted and complemented, trying to portray the complex connections between parameters in a radial plot (Resch et al., 2020; Aeby et al., 2023; Aeby & Wyss, 2021).

Approaches and challenges

So far, the options to evaluate silage quality include: 1. Assessing the ensiling success according to sensory evaluation, analysis of acids including the pH value; 2. Determining the nutritional content by laboratory analysis; 3. Deciding on a quality class; 4. Evaluating the palatability (Schmidt et al., 1972).

In principle, the options have barely changed. However, the range of analysis parameters has broadened, and complex statistical calculations make it possible to draw better the connection between chemical analysis and animal performance parameters (Tharangani et al., 2022; Tharangani et al., 2021; Andrighetto et al., 2018). When evaluating silage quality nowadays, several external factors must be considered, which have changed the agricultural holdings in Western Europe since the 1980s when many of the criteria were defined. Some of them are listed here:

- Silage is no longer just a winter feed but has become a year-round staple feed for dairy cattle, ideally in stable rations, and for biogas plants.
- Due to breeding for cold tolerance, maize silage, in high chop quality, has become an important component of cattle feeding rations even in the far North.
- Different forage species, cultivars, other crops, and by-products are ensiled frequently (not evaluated before).
- Increased requirements for high-quality feed due to increasing genetic efficiency in the dairy cow sector.
- Dominance of total mixed rations (TMR) for stable-fed animals.
- The increased mechanization has allowed for huge horizontal silos with several meters height of silo piles.
- High power for the harvest exceeds the capacity to compact the harvested forage when filling the silo.
- Concrete bunker silos have become the predominant silo type with largely improved silofilm qualities.
- Biological silage additives are the preferred type of additive.
- Easy data collection (e.g., yield, dry matter) due to progress in information technology.
- Routine laboratory analysis of basic feed value parameters is much faster and cheaper thanks to calibrations with near-infrared reflectance spectroscopy.

Conclusions

Which implications have these had on silage quality and its capturing?

- Short ensiling durations are more frequent to avoid changing the ration component, which affects silage hygiene and stability.
- A shift from butyric acid fermentation towards aerobic instability in problem silages and in TMR.
- Problems with static and pressures in high silo piles.
- More theoretical knowledge available.

Silage quality expresses the feed's practical value and shall finally reflect the animal response. For the routine day-by-day silo control by farmers and extensionists, reasonable and ideally simple indicators are needed. They can be supplemented by laboratory analyses if serious trouble is predicted or encountered. These complex silage assessment criteria, target figures, and possible thresholds are expanded on in the related five abstracts "Silage TROUBLESHOOTING – Part I-V" (this volume), reflecting the current state of the art system, which can be built on in future practice-oriented research.

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All references are provided on request.

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Detecting Aerobic Changes in Silages and Silage-Based Rations on Farms and in Laboratory Scale Silos – Part A: Indicators

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Keywords: aerobic deterioration, indicators, pH change, temperature increase, visual assessment

Introduction

Silage is a link in the chain of food production. In the food sector, shelf life is a central question. Not intended changes affecting taste, nutritional value, and microbiological quality are undesirable and considered as spoilage. The product must be withdrawn from circulation immediately. Deterioration processes in food and feed are comparable, driven by similar factors: physicochemical changes, enzymes inherent in the food, microorganisms, nutrient and oxygen availability, pH, humidity, and temperature are the most important ones (Ghasemi-Varnamkhasti *et al.*, 2018). Nowadays, the highest risk of silage deterioration and related losses is in the feed-out phase of silos, such as bunker silos and piles.

In the last decades, silage science has established that the temperature increase of silages above ambient (+ 1, 2, or 3 K) is the criterion for the onset of aerobic deterioration (Driehuis *et al.*, 2001; Huisden *et al.*, 2009; O'Kiely & Muck, 1992). Although heating is a sufficient sign of aerobic deterioration, it does not necessarily occur. Silage and related products can spoil without warming upon exposure to air.

The following briefly outlines the historical development of the temperature criterion and some principal processes of aerobic changes. Based on this, the leading indicators are presented, and both simple (Part A) and the latest technical recording possibilities are discussed (Maack & Martens, Part B, this volume).

Microbial processes in silages upon exposure to air and their detection

Lack of oxygen and a high concentration of dissociated organic acids inhibit the activity of most of the deleterious microorganisms in the sealed silage (conserving effect). Once the silo is open again, gas exchange supplies oxygen to bacteria and fungi. In most cases, yeasts are the pioneers of the deterioration cascade due to their acid tolerance (Beck & Gross, 1964). Secondly, yeast species can oxidize lactic acid via the glycolytic pathway and tricarboxylic acid cycle, releasing heat and producing CO₂ and water as end products (Rooke & Hatfield, 2003). In order to quantify aerobic losses in grass silages, Honig (1990) measured the amounts of CO₂ released from a 1 L test cylinder containing a defined amount of grass silage. In order to simplify the measurement procedure, Honig implemented a test series recording both CO₂ concentrations and temperature development during several days with grass silages of a wide range of dry matter (DM) content. Thus, he related losses to temperature rise, considering differing heat capacity and conductivity. However, the recorded temperature increases never achieved the theoretical ones calculated by McDonald *et al.* (1991), assuming complete oxidation of lactic acid. On the other hand, in a silage stack, the source of CO₂ has to be identified, whether it originates from the pores of anaerobic storage or from new microbial activity (Shan *et al.*, 2019). Nevertheless, the easily detectable “temperature” has become the state of the art for detecting aerobic instability of silages.

When using temperature increase as the single indicator of aerobic instability, it has to be taken into account that different respiratory pathways exist, such as alternative oxidase in fungi, accompanied by less heat development (Joseph-Horne *et al.*, 2001).

That is one possible reason why signs of spoilage were detected in different types of silage without or with minimal temperature increase (Martens *et al.*, 2022).

Another indicator of microbial respiratory activity is O₂ consumption, which is still challenging to measure (Shan *et al.*, 2021; see Part B).

When lactic acid is consumed, silage is gradually neutralized. Thus, an increasing pH value also indicates aerobic changes. However, depending on the ensiled feed type, its buffering capacity, and other factors, pH may react in a delayed manner to actual acid decomposition (Martens, 2006).

Another physical factor is the formation of water during the respiration. Since common humidity loggers are unsuitable for this application, so a simple visual assessment with scores from 0 to 4 (no condensation to completely wet) is recommended (Martens *et al.*, 2022). Weight losses cannot be determined accurately due to the condensed water in the container, which interferes with weighing the available feed. However, weight differences are an additional information.

Besides all measurements of physical factors, the sensory evaluation of fungal growth is a valuable information source. A visual appraisal of yeast colonies and mold agglomerations in the feed on a scale from 0 to 4 (no signs to totally infested) provides the only indication of aerobic spoilage in some cases, especially in the early stages (Martens *et al.*, 2022).

Conclusions

In order to adequately describe aerobic changes in silages and silage-based rations, it is recommended that the range of indicators be expanded beyond temperature recording. These are easy to detect without special laboratory equipment: pH changes, condensation, and fungal infestation by visual appraisal. Furthermore, considering the whole temperature curve and the maximum temperature difference gives valuable hints on the extent of microbial spoilage. This video illustrates the simple setup as an inexpensive method to detect signs of aerobic changes easily and accurately using total mixed ration as an example: <https://lsnq.de/tmraerobicstabilitytest>. It considers the effects of microbial activity in its different characteristics: heat development and condensation, degradation of acids, development of fungal colonies, and mass losses.

For detailed monitoring of the processes by changes of physical parameters, a sophisticated setup is presented in Part B (Maack & Martens, this volume).

For further aspects on farms, see this volume, Maack et al. (2025) and Martens & Resch (2025).

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Screening Heterofermentative Lactic Acid Bacteria for Osmotolerance

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Keywords: growth medium, high dry matter, *in vitro*, protocol, wilting

Introduction

Rapidly increasing the dry matter of forages prior to ensiling involves the benefits of reducing proteolytic processes, concentrating nutrients, and preventing effluent. On the other hand, high dry matter silage is rather prone to aerobic deterioration as the required compaction is more difficult to achieve, thus leaving more pore volume for air movement. Obligately heterofermentative lactic acid bacteria (LAB) provide an opportunity to improve aerobic stability. The question is how to screen strains, which are especially suitable for high dry matter (DM) conditions, where increased osmotic pressure prevails. A method was presented for homofermentative strains, effectively simulating high osmotic pressure by using increased concentrations of sugars in a culture medium *in vitro*. It has been inconclusive for two heterofermentative strains so far (Martens *et al.* 2024). Here, a range of different heterofermentative species and strains was evaluated *in situ* and *in vitro*. The pH of inoculated grass at different DM levels at an early stage of ensiling was compared to the pH of an inoculated culture medium, being low or high in osmolality respectively in order to validate the *in vitro* approach.

Materials and methods

In 2024, five different products were tested, four of them containing different strains of *Lentilactobacillus buchneri* exclusively (LAB1-4), and one containing a mixture of *L. buchneri*, *L. diolivorans* and *Lactocaseibacillus rhamnosus* (LAB5). Between the end of May to mid-July, grass was wilted to 35-49 % DM as target at five different locations in Germany. In addition to an uninoculated control, the chopped forage was treated with the silage additives in triplicates (dosage as recommended by the manufacturers). *In vitro*, MRS broth (regular osmolality) and "high sugar" MRS broth (high osmolality; HS) were inoculated in triplicates. HS contained MRS as dehydrated culture media (54 g) plus 100 g glucose and fructose each, along with 28 g KCl dissolved in 1.0 L distilled water, and was adjusted to pH 5.7 using NaOH (Martens *et al.* 2024, modified). The pH development, both in the grass silage (after 0 and 5 days), and *in vitro* (after 0, 24, 36 and 48 h), was recorded to evaluate the LAB activity under these conditions of low and high osmotic pressure. A period of five instead of three days was chosen with heterofermentative LAB in contrast to the trials with homofermentative LAB as the former generally produce less lactic acid, resulting in a slower pH decrease (von Wright & Axelsson 2019). A linear regression analysis was performed.

Results and discussion

The actual achieved DM ranged from 30.7-52.6 % in the grass silages. Except for one location, the uninoculated control behaved similarly to the inoculated grass regarding the pH decrease during the first 5 days of ensiling, in relation to the actual DM content. This means that it was generally difficult to separate the effect of this group of inoculants from the epiphytic microflora in the initial phase of ensiling. There was a significant correlation between DM content to pH ($r^2 = 0.60$, $P < 0.001$; Fig. 1a). However, the slope was very weak compared to silages inoculated with homofermentative strains (Fig. 1 a, b; Martens *et al.*, 2024). *In vitro*, the initial inhibition of fermentation was similar for three out of five products at regular and increased osmolality (Fig. 2). LAB3 and LAB5 showed higher acidification at regular osmolality than the other inoculants, but were also impeded in HS. However, after 36 h, all the strains had overcome the initial impediment (Fig. 2). This study confirms other results that increased DM (studied ranges from 23-41 % DM in maize and grass silages) has minor impact on the fermentative capacity of obligately heterofermentative in contrast to homofermentative LAB (Hu *et al.*, 2009; Driehuis *et al.*, 2001).

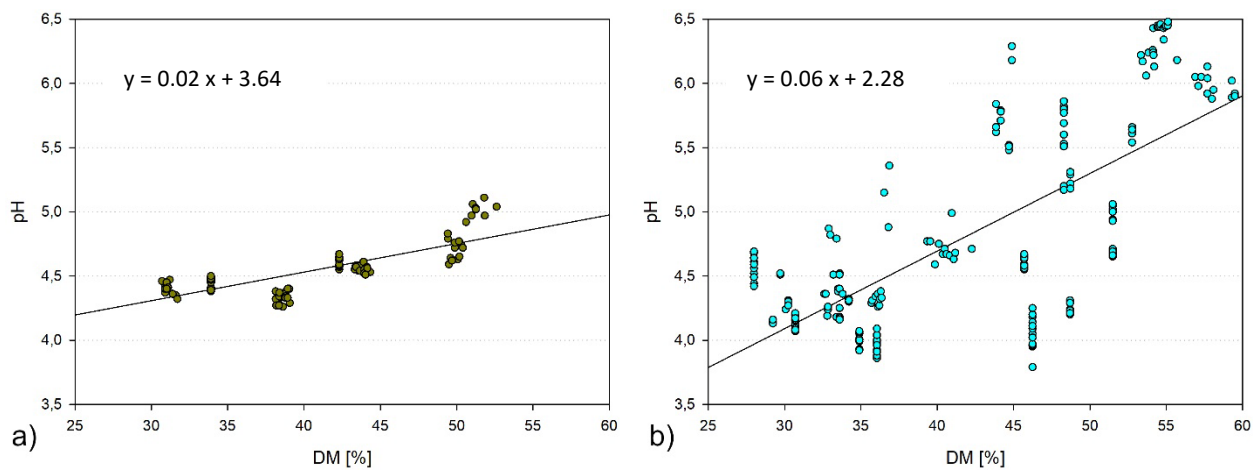


Figure 1. Linear regression between DM content and pH value of grass silages inoculated a) with heterofermentative LAB, b) with homofermentative LAB (Martens et al. 2024). [Commas in the graphs are decimal separators.]

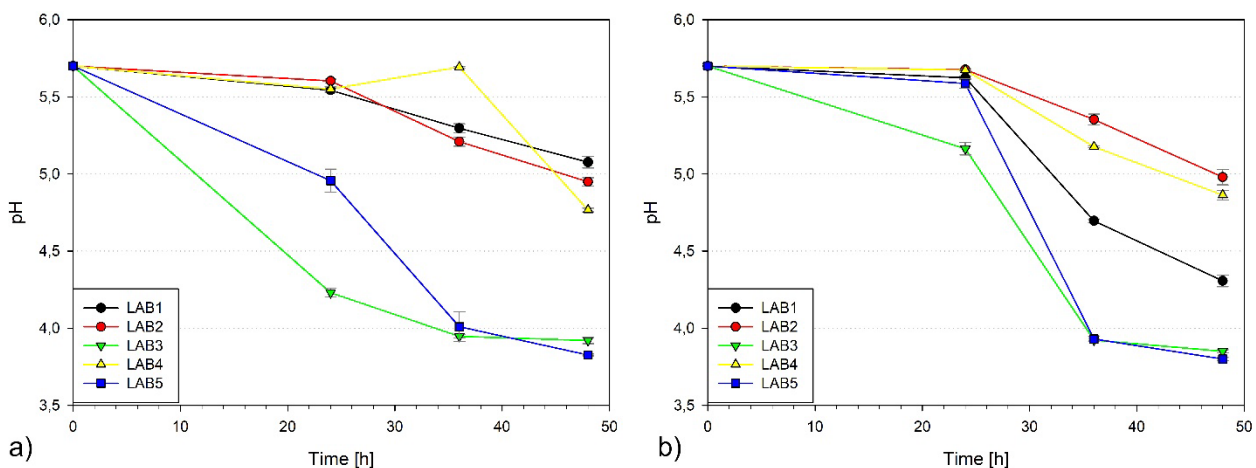


Figure 2. pH development *in vitro* for 48 h. Media inoculated with 5 different additives (LAB1-5). a) MRS, b) High sugar medium with high osmolality. [Commas in the graphs are decimal separators.]

Conclusions

The study revealed that the metabolic activity of heterofermentative LAB in the initial phase of ensiling is barely affected by high osmolality prevailing at high DM conditions in wilted forages.

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Silage Troubleshooting – Part II: Evaluation of Sensory Aspects

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Keywords: fermentation failures, hygienic status, organoleptic assessment, spoilage

Introduction

High performance of dairy cattle requires daily monitoring of silage quality to ensure consistent feed intake, performance, and animal health. Different chemical and microbiological laboratory methods are the standard for silage evaluation (Cherney & Cherney, 2003). In German-speaking Western European countries, essential chemical parameters (see Part IV, Schneider et al., this volume) and microbiological parameters (see Part V, Wagner et al., this volume) are examined in grass silage routine assessments. There is always an unavoidable delay between the time of sampling and the laboratory report, which is a disadvantage for timely troubleshooting. The development and application of sensory evaluation methods in practice have enabled farmers and employees to continuously monitor their silage quality on-site with high certainty. One of the early systematic sensory methods evaluated odor, structure, and color with different weightings to classify silage quality into five levels (Zimmer, 1959). Current sensory evaluation methods, such as DLG (2004), Resch et al. (2020), or Aeby et al. (2023), record more aspects of silage quality step by step using a simple form in a similar way to the past. However, the level of silage deficits is now valued negatively as deduction points: the more deduction points, the poorer the silage quality.

In addition to grass silage assessment, the DLG evaluation key (DLG 2004) also includes a procedure for maize silage and hay. The Austrian key, according to Resch et al. (2020), also contains valuation instructions for haylage (DM > 50 %) and straw. The evaluation procedures described below for the three countries for grass silage are basically similar in structure, although there are specific differences in their individual assessment sections.

The principle of today's sensory assessment for grass silage

Nowadays, on-farm sensory silage evaluation methods in German-speaking countries are designed to systematically classify the quality of the sampled silage with the aid of sensory parameters. In principle, the quality of the silage is determined in three main areas. 1) Classification of the value of green forage, 2) Sensory evaluation of the silage, 3) Energy loss due to preservation and contamination by the soil.

1) Value of green forage: Depending on the scheme, three to nine grassland types are distinguished, whereby the proportion of grass plays an important role. In practice, only a simple classification of grassland types is usually possible from a finely chopped silage sample. For a more detailed grassland categorization, on-field inspection is required prior to ensiling. The phenological stage of the plants in the first growth is divided into seven stages, from shooting to seed maturity. According to DLG (2004) and Resch et al. (2020), *Dactylis glomerata* and *Taraxacum officinale* are categorized. Aeby et al. (2023) also classify *Alopecurus pratensis*, *Phleum pratense*, *Trifolium pratense*, and *Medicago sativa*, depending on their presence. Re-growths are divided into four to five stages, depending on weeks from the last harvest date. This information helps to assign the energy value of the green forage before harvest according to national forage value tables.

2) Sensory evaluation of silage: The first aim is to locate the cause of problems using sensory assessment. The main focus is on odor, as silage deficiencies (troubleshooting areas) - suboptimal fermentation, heating, yeast, mold formation, and putrefaction - can be detected by the smell in most cases. Optimally fermented grass silage is free of butyric acid and some other strange odors and should also smell pleasantly acidic and aromatic. The stronger the butyric acid (rancid butter, sweaty), ammonia (smell of stable, urine), alcohol (volatile, yeasty) or ester (adhesive-, acetone-like or fruity), smell of roasting (bread-like, burnt, tobacco-like), mold (musty) or putrefaction (rotting smell), but also too much acetic acid (acidic odor), the more deduction points are awarded for any of these shortcomings. Grass silages of autumn harvest are an exception to this. They may have no or weak fermentation due to cool temperatures, so their color is fresh green, and the smell is often grassy.

The optimum silage color is olive green to slightly brownish, depending on the original forage. Noticeable deviations in silage color from the optimum usually indicate undesirable changes in quality in combination with aspects of odor, consistency, or heating. Silage heating as an effect of low aerobic stability (see Part II, Martens et al., this volume) is an important area of regular sensory silage evaluation, especially in the feed-out phase. The silage should not be greasy or dirty; otherwise, deduction points will result.

Determination of the DM content by manual pressing or wringing is very simple and reliable for silage, especially below 50 % DM. The test for insufficient acid formation is assessed by pH measurement (indicator paper or pH meter) to assess anaerobic stability. DM and pH correlate positively, i.e., the critical pH value increases with an increase in DM: pH <4.0 for DM ≤20% or pH <5.0 for DM >45%. Depending on the extent to which the pH value is above this level, up to 4 resp. five deduction points are given for the pH value. If necessary, further relevant parameters like management (see Part I, Maack et al., this volume) and poisonous plants are included for a final assessment.

3) Evaluation of energy loss: After the sensory evaluation, the deduction points are added up. Depending on the number of points, the result is classified into 5 grades (DLG, 2004; Resch et al., 2020) ranging from very good (< 2 points) to very poor (> 8 points). Each class is assigned a reduction in energy value compared to green forage in MJ kg DM⁻¹. Depending on the degree of soiling, there are additional energy deductions. In the Swiss system, according to Aeby et al. (2023), certain correction factors for NEL, NEV, APDE, and APDN are derived depending on the conservation quality to calculate a corrected nutritional value of the grass silage. However, the energy content of the on-farm grass silage assessment is the final result of the quality calculation via the processes of harvest, ensiling and feed-out phase.

Conclusions

In addition to chemical and microbiological analysis, systematic sensory evaluation of silage on-farm is recommended to take measures very quickly for the daily feeding routine, such as discarding critical parts or adapting the feed-out rate and the ration for the different livestock categories. If anaerobic or aerobic spoilage is detected, the evaluated silage may no longer be fed to livestock. Evaluated sensory aspects allow conclusions to be drawn about the causes of silage problems. Sources of deficiencies can be detected more easily and considered for the next ensiling campaign. There is a need for modified evaluation schemes for other forages, such as pure legumes, as they ensile differently to grasses. However, the sensory evaluation schemes described for grass silage, like the other four parts of silage diagnostics, are part of a comprehensive silage assessment system (Martens et al., 2025).

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Enhancing the Use of Food Waste Through Ensiling

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Keywords: food waste, silage, aerobic stability, circular bioeconomy.

Enhancing circularity is the key to developing sustainable livestock and food production systems. Livestock have been scrutinized for their broad environmental footprint, whereas their contribution to circular food systems is frequently overlooked. Reducing food loss and waste (FLW) is central to improving the circularity of food production systems as 13.2% of food is lost between harvest and retail, with an additional 19% wasted in households, food services and the retail sector. Food loss and waste is responsible for approximately 7% of global greenhouse gas (GHG) emissions and utilizes about 30% of global agricultural land (United Nations 2024). Optimally FLW should be directed towards consumption and sustained in the food chain, but cosmetic defects and unforeseen challenges such as disease, drought, floods and spoilage often preclude this pathway. Alternatively, FLW can be directed for use as livestock feed, a practice that reduces the environmental footprint of livestock and ensures that agricultural land continues to be used to produce food. This practice also avoids the GHG emissions that arise if FLW is landfilled. In fact, most by-products and residues arising from industrial food processing are already used as livestock feed. However, some FLW sources are high in moisture and rapidly spoil, necessitating that they either be dried or fed to livestock shortly after collection. Drying of high-moisture FLW is often impractical as it increases feed costs and generates GHG emissions. Ensiling may be a viable approach to the ensuring the preservation of high-moisture FLW. However, ensiling of FLW has challenges due to its variability in chemical composition, moisture content and unpredictable supply chains. Mixing of high moisture FLW such as fruits and vegetables with dry sources such as bakery waste can generate FLW mixtures suitable for ensiling. Most retail sources of FLW are high in water soluble carbohydrates, and generate high lactic acid yields, but some can also generate excessive amounts of ethanol during ensiling. The batches of FLW arising from retail and food services are generally small and vary considerably throughout the year, making it challenging to generate FLW mixtures with predictable nutrient contents and of sufficient volume for use in large scale ruminant production systems. Furthermore, given the diversity of FLW sources, some may not be approved by regulatory agencies for use as livestock feed. Care must also be taken as FLW that has started to decompose may contain mycotoxins or other anti-nutritional factors that adversely impact silage quality and threaten livestock health. These challenges need to be regionally assessed, and ensiling practices need to be designed at a scale that meets the needs of local livestock producers. Silage inoculants and the development of alternative small scale ensiling practices could further increase the feed value and use of FLW as silage. Developing ensiling practices that enhance the use of FLW could be an avenue to reducing the carbon footprint of livestock and increasing their contribution to the circularity of agricultural systems.

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Characterization of Density and Fermentation Variables in Silos with Oxygen Barriers: A Farm Survey in Argentina

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Keywords: Fermentation variables, mycotoxins, oxygen barrier, silage density.

Introduction

In Argentina, more than 70% of silage is stored in silo bags (CACF, 2024). This is primarily because drive-over pile silos (DOPS) had low compaction ($<180 \text{ kgDM/m}^3$) and either poor sealing or no covers (~20% uncovered). The growth of farms scales has increased the need for DOPS to reduce plastic use and storage area. DOPS require high filling rate, good compaction ($>240 \text{ kgDM/m}^3$), and quick and efficient sealing. Oxygen barrier films (OB) can help to reduce losses or mitigate the suboptimal silage-making process. OB, by limiting oxygen permeability, delays the onset of aerobic deterioration and microbial growth (including the mycotoxigenic ones). This helps maintain feed quality over extended periods in farm-scale silage where is consumed over several months (Borreani & Tabacco, 2008). Mycotoxins are eluted, degraded, or adsorbed during fermentation, but certain poorly preserved silages are prone to additional mycotoxin production during the stable phase due to oxygen ingress, leading to extremely high toxin levels (Vandicke et al., 2021). Slow or delayed filling of DOPS, low packing, deficient or not sealing and damaged plastic wrap can promote mold proliferation and mycotoxin production before opening and usage (Ogunade et al., 2018). The aim of the present study was to characterize whole-plant corn silage DOPS with low and significant differences in density between the core and the top, covered with OB, on fermentation variables and mycotoxins concentration.

Materials and methods

During 2024, we selected three DOPS (~200 ha each), stored for >100 days from three farms in Argentina (Córdoba and Buenos Aires Provinces), all covered with OB (SealPlus 120 μm One-step). Each silo was subsampled into three depth sections on the exposed face: 25 cm from the top (25CM), 50 cm from the top (50CM), and the core, approximately 150 cm from the floor (CORE). The selected silos were required to have significant differences in density (kgDM/m^3) among depth sections. Samples ($n=27$) were collected using a master forage probe to measure silage density at each sampling point. Dry matter (DM, %), pH, lactic acid (%DM), acetic acid (%DM), ammonia-N (%CP), protein solubility (%CP), acid detergent insoluble crude protein (ADICP, %CP), ash (%DM), and sugar WSC (%DM) were analyzed using NIRS, while mycotoxins (Aflatoxin-AFB1, Zearalenone-Zea, and Deoxynivalenol-DON) were determined by RIDASCREEN® Mycotoxin ELISA tests (R-Biopharm) at Rock River Laboratory Inc. (Santa Fe Province, Argentina). Density was determined by dividing fresh weight by probe volume and converting it to dry matter (kgDM/m^3) using laboratory data. The dataset was subjected to analysis of variance (ANOVA) and LSD tests using Infostat 2020.

Results

Densities were 80.88 kgDM/m^3 , 122.96 kgDM/m^3 and 226.32 kgDM/m^3 for 25CM, 50CM and CORE respectively (SE= 12.51; $p\text{-value} < 0.001$). The CORE had the highest density but was below 240 kgDM/m^3 . Most fermentative variables showed no significant differences between depths, despite density variations and the higher density of the CORE. No differences were found for pH, lactic acid, acetic acid concentration. Likewise, both concentrations were numerical highest in the CORE, but lactic/acetic ratio was lower (25CM= 2.42, 50CM= 1.70, CORE= 1.47), suggesting a slow and heterofermentative fermentation at this depth. Xu et al. (2024) reported different results in *Pennisetum giganteum*, where higher densities were associated with more lactic acid, a higher lactic/acetic ratio, and lower pH values. The lack of differences in pH values among depths ($P = 0.24$) indicates a uniform stabilization of fermentation across the silo depths. Ammonia-N (%CP) and protein soluble (%CP) were significantly higher for CORE than 25CM and 50CM respectively ($P < 0.05$). Xu et al. (2024) also found higher ammonia-N levels in low-density silage. Protein breakdown may be also because of slow and heterofermentative fermentation at this depth. Rapid and efficient fermentation, driven mainly by homolactic bacteria, is known to reduce proteolysis (Borreani et al., 2018). ADICP (%CP), ash (%DM), and sugar WSC (%DM) did not show significant differences by depth ($P > 0.05$), indicating that oxidative damage was minimal, and density did not have negative effects on these variables. Mycotoxin was marginally significant difference in AFB1 ($P=0.053$). AFB1 concentrations were below the EU maximum limit for adult ruminants (20 ppb) and the average value of 15.82 ppb reported by Ogunade et al. (2018) for Argentina, although the CORE had higher values than 25CM and 50CM. ZEA and DON concentration did not differ significantly by depth ($P > 0.05$). However, ZEA levels differed between silos. ZEA levels were below the EU maximum limit (500 ppb) for 25CM and 50CM but were higher in the CORE. ZEA values at 25CM and 50CM were also below the average of 436.4 ppb reported by Ogunade et al. (2018) for Argentina, while the CORE exceeded this value. DON was homogenous among the depth. The higher concentrations of mycotoxins in the CORE could be due to contamination and a stressful environment for molds, possibly caused by delayed covering with low oxygen levels.

Table 1. Characterization of fermentation variables and mycotoxin at 25 cm from the top (25CM), 50 cm from the top (50CM), and ~150cm from the floor (CORE). The 25CM and 50CM and CORE are means of three replicates of the three silos (n=9 by depth of DOPS).

Variable	25CM	50CM	CORE	P-Value
Dry matter, %	45.06	45.11	39.46	0.445
pH	4.13	4.13	4.01	0.239
Lactic acid, %DM	2.88	2.56	3.29	0.441
Acetic acid, %DM	1.19	1.51	2.24	0.096
Ammonia-N, %CP	7.15 ^b	8.11 ^b	11.21 ^a	<0.001
Protein solubility, %CP	62.27 ^b	61.54 ^b	68.34 ^a	0.003
ADICP, %CP	8.11	9.26	7.77	0.372
Ash, %DM	6.1	5.74	5.53	0.352
Sugar WSC, %DM	3.96	4.1	4.69	0.149
AFB1, ppb	7.28 ^b	7.37 ^b	13.72 ^a	0.053
ZEA, ppb	375.01	311.64	699.97	0.156
DON, ppm	0.93	0.81	0.96	0.798

Means in the same row with different letters differ significantly ($P < 0.05$).

Conclusions

Comparison across depths suggests that effective sealing with OB mitigated the effects of differences and low density in the upper layers. The lack of significant differences in most of the variables suggests consistent fermentation across the silo but highlights the need for better compaction and filling rate. The CORE exhibited signs of delayed fermentation, possibly due to the large size of DOPS and the time required for completion and sealing. Is important to emphasize that achieving good DOPS involves maintaining a healthy crop, preparing an appropriate site, using adequate equipment to complete ensiling as quickly as possible, covering promptly, use additives and ensuring the silo remains sealed until use.

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Relationship Between Nutritional Variables of Pre- and Post-Ensiled Corn Crops: A Meta-Analysis

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Keywords: fresh corn, ensiling process, nutritional variables, silage.

Introduction

The nutritional quality of corn silage is influenced by many factors, including plant maturity, cutting height, and hybrid type. Higher cutting heights can increase digestibility and total digestible nutrients without affecting fermentation quality (Aoki et al., 2013). The new technology like NIRS in the choppers allows the real-time analysis of crop components during harvest, enabling better decision-making before and during the silage-making process. This facilitates the segregation and homogenizing quality of stored material or improvement of the target quality for storage through real-time adjustments, such as chopping by zones, changing lots, or adjusting platform height (i.e., more height, less NDF). Working with target quality levels according to the intended use of the silage is a necessary step in precision livestock farming. The challenge is determining the quality that fresh forage must have to achieve the target quality after the silage process. However, little is known about the accuracy of predicting silage composition from crop data. Udén (2018) suggests that it is crucial to investigate which feed components, besides water-soluble carbohydrates (WSC), may be utilized or modified by microorganisms during silage fermentation. Despite the available research, there remains a scarcity of definitive references that clarify the relationship between the nutritional quality of the corn plant and the quality of the resulting silage. This meta-analysis aims to systematically synthesize findings on the correlation and variation between the quality components of pre-ensiled and post-ensiled corn crops.

Materials and methods

We searched literature to identify experiments that analyze nutritional variables of corn crops before ensilage (pre-ensiled), and after ensilage (post-ensiled). We utilized multiple search engines, employing keywords such fresh corn for silage, pre-ensiled corn, ensiling process corn silage. A total of 37 publications were initially identified (1985 to 2023); however, only 12 articles were included in the final dataset. For articles with a single pre-ensiled analysis but multiple post-ensiled data points, the post-ensiled values were averaged to create a one-to-one dataset. This process resulted in a total of 42 treatment containing information on pre- and post-ensiled quality components: dry matter (DM, %; n= 40), crude protein (CP, %; 42), acid detergent fiber (ADF, %; n= 32), neutral detergent fiber (NDF, %; n=42), starch (STCH, %; n=35), sugar (WSC, %; n=28) and ash (%;n= 23). Relative variability (VAR) between pre- and post-ensiled values was calculated by dividing the post-ensiled parameter by the pre-ensiled parameter, subtracting 1, and multiplying by 100 to express the result as a percentage (%). Negative and positive VAR indicate a decrease and an increase in post-ensiled values, respectively. Data were subjected to linear regression analysis for each variable between pre-ensiled (x) vs pos-ensiled (Y) (InfoStat, Di Rienzo et al., 2020).

Results

Table 1. Relative variation (VAR; %) of each post-ensiled relative to its pre-ensiled variable (mean $\pm$ SD). R²: coefficient of determination for estimating each post-ensiled variable (Y) as a function of the pre-ensiled variable (x), expressed as $Y = \text{intercept} + (\text{slope} \cdot x)$; *P* value <0.05 indicate linear correlation significative.

Variable	VAR (%) Mean $\pm$ SD	R ²	Intercept	Slope	<i>P</i> value
DM, %	-0.74 $\pm$ 4.57	0.970	2.560	0.900	<0.0001
CP, %	11.76 $\pm$ 16.36	0.084	5.712	0.358	0.0630
ADF, %	4.81 $\pm$ 15.05	0.629	-5.282	1.240	<0.0001
NDF, %	-2.96 $\pm$ 16.58	0.613	-12.184	1.224	<0.0001
STCH, %	15.21 $\pm$ 20.81	0.954	1.597	1.057	<0.0001
WSC, %	-79.79 $\pm$ 28.01	0.014	2.366	-0.038	0.5456
Ash, %	11.29 $\pm$ 15.85	0.422	1.274	0.865	0.0008

DM and NDF showed a significant correlation ($P < 0.05$) from pre-ensiled to post-ensiled material with negative VAR (decrease post-ensiled), likely due to oxidative transformations increasing water concentration during air elimination. But some studies suggest that DM is higher post-ensiling (Guyader et al., 2018b). NDF may decrease due to the degradation of labile fractions (hemicellulose and cellulose) into simple carbohydrates during fermentation, while ADF remains stable (Seale et al., 1986). WSC also showed a negative VAR without significant correlation ($P > 0.05$). WSCs are quickly transformed into organic acids like lactic acid that enhances digestibility compared to pre-ensiled (Ferraretto et al., 2014). ADF, STCH, and Ash showed a significant correlation ($P < 0.05$) with positive VAR (increase post-ensiled). The ADF increases due to the reduction of labile NDF components, while STCH, requiring over 180 days to degrade, improves digestibility over time (Ferraretto et al., 2018). Ash concentration rises as other components diminish, serving as an indirect measure of spoilage and soil addition (Borreani et al., 2018). CP showed positive VAR but no significant correlation. Protein loss through ammonia production and proteolysis is a multifactorial phenomenon

during the ensiling process (Borreani et al., 2018), including the degradation of nitrogenous compounds and changes in fermentation conditions.

Conclusions

This meta-analysis highlights the importance of real-time decision-making during silage production to ensure desired outcomes. The significant correlations between pre-ensiled and post-ensiled components, such as DM, NDF, ADF, and STCH, demonstrate that silage quality can be actively managed. Utilizing tools like NIRS technology during harvesting allows producers to adjust cutting height, crop segregation, and other parameters to meet target quality levels. This proactive approach ensures consistent silage quality tailored to livestock nutritional needs. Future analyses should integrate the influences of storage duration, bacterial inoculation, and the achieved density within the silo into the evaluation.

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Estimation of Silage Component Traits in Maize Using Near-infrared Reflectance Spectroscopy

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Keywords: Silage, Maize, NIRS

Introduction

Maize silage, developed through anaerobic fermentation of whole maize plants, serves as a high-moisture forage and is increasingly recognized as a vital feed component in modern dairy farming systems. Ideal silage maize hybrids exhibit attributes such as elevated dry matter production, enhanced digestibility and energy levels, low fiber for better intake, substantial protein concentration, and optimal harvest-stage moisture to facilitate effective fermentation and storage (Karnatam et al., 2023). Estimating silage quality is crucial in crop breeding programs, enabling plant breeders to develop superior hybrids. Although traditional wet chemistry techniques yield accurate quantification of forage components, they are labor-intensive, time-demanding, and require substantial resources. Near-infrared reflectance spectroscopy (NIRS) presents a rapid, non-invasive, high-throughput economical solution, enabling concurrent analysis of various quality parameters with minimal preprocessing (Shenk & Westerhaus, 1994). Therefore, this study focuses on developing calibration and validation models for various silage component traits in maize.

Materials and methods

An F1 maize hybrid population consisting of 354 hybrids was developed using diallel crosses and NCD II designs, involving 25 subtropical maize inbred parents from PAU and 17 temperate-background maize inbred parents from CIMMYT. The hybrids were evaluated in two replications across two locations from February to May 2024.

Over 1600 maize plants were harvested at the silage maturity stage, processed by chopping, oven-drying, and grinding, and subsequently sieved to a 1 mm particle size using a Wiley mill. The spectral signatures of these samples were captured using FOSS XDS Rapid Content Analyser® overall spectral range of 400 to 2500 nm. The samples were analyzed using the WinISI software suite and a pre-existing global maize calibration equation (listed in the NIRS microsite). The primary traits predicted included nitrogen (N), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), metabolizable energy (ME), and in vitro organic matter digestibility (IVOMD).

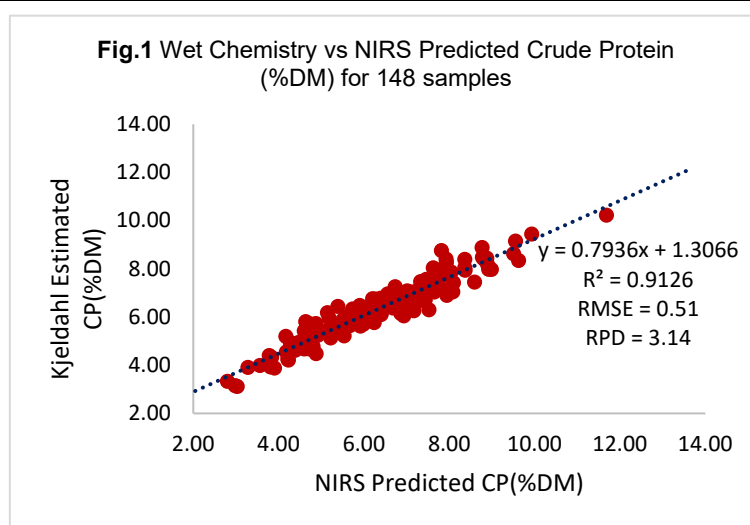
To validate crude protein predictions, 148 representative samples were selected from the initial dataset of 1,600 and analyzed for crude protein content using the Kjeldahl method (AOAC 955.04). The resulting reference data were used to enhance the existing calibration and develop an improved prediction model specifically tailored for silage-stage maize. The metrics of standard error of calibration (SEC) and validation (SEV), and coefficient of determination for calibration (R^2 cal) and validation (R^2 val) were used for assessing prediction accuracy.

Results

Kjeldahl analysis of 148 samples resulted in nitrogen concentrations (N%DM) ranging from 0.26% to 1.77%, which, using the standard conversion factor ($CP\% = N\% \times 6.25$), correspond to crude protein contents (CP% DM) ranging from 1.63% to 11.06%. The R^2 values showed high predictive strength, with calibration and validation scores ranging from 0.96 to 0.97 for N, NDF, and ADF; 0.92 to 0.93 for ME and IVOMD; and 0.86 to 0.87 for ADL (Table 1). Associated SE values spanned 0.05 to 1.44 during calibration and 0.05 to 1.62 in validation (Table 1). These metrics affirm the reliability of NIRS models for estimating silage quality parameters in maize. Figure 1 illustrates the strong correlation between crude protein values predicted using NIRS and those determined through Kjeldahl analysis, validating the model's accuracy.

Table 1: Silage quality traits of F1 hybrids and inbreds predicted using the improved global maize calibration equation along with the crude protein content estimated using Kjeldahl analysis. (N=nitrogen, NDF=, ADF, ADL, ME, IVOMD, n=number of samples, stdev=standard deviation, min=minimum, max=maximum, SEC=standard error of calibration, SEV=standard error of validation, cal=calibration dataset, val=validation dataset)

Model metrics of improved global maize calibration equation									
Constituent	n	Mean	Stdev	Min	Max	SEC	R ² _{cal}	SEV	R ² _{val}
N(%)	813	0.83	0.27	0.05	1.61	0.05	0.97	0.05	0.96
NDF(%)	663	69.27	6.43	49.94	88.51	1.14	0.97	1.23	0.96
ADF(%)	667	38.45	5.58	21.63	55.29	0.92	0.97	1.01	0.97
ADL(%)	666	3.31	0.94	0.47	6.15	0.34	0.87	0.36	0.86
ME(MJ/Kg)	654	8.35	0.89	5.68	10.93	0.23	0.93	0.26	0.92
IVOMD(%)	659	56.17	5.58	39.52	72.24	1.47	0.93	1.66	0.91
Results of Kjeldahl analysis:									
Nitrogen content (%)	148	1.02	0.25	0.26	1.77	0.05	0.95	0.08	0.90



Conclusions

With a throughput of analyzing 150 samples per day, NIRS can potentially enable breeders to select and evaluate a large population of lines for quality traits with high accuracy and cost-and resource-efficiency.

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Associative Effect of Ingredient Sources on the Partial Mixed Ration and TMR Silages for Feedlot Beef Cattle Diets in Brazil

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Keywords: Partial mixed ration silage, TMR silage, Effluents, Water holding capacity, Wet distillers bran plus solubles.

Introduction

Corn-based ethanol industry evolved in Brazil reaching 22 plants in 2024, producing together 5 million ton of DDG (dried) and WDG (wet) coproducts. Besides the huge increase of DDG for ruminant and monogastric diets, it is still there the offering of wet distillers grains with solubles (WDGS) as a diet ingredient, mostly for finishing beef cattle. Its typical high moisture content (60-65%) and high solubles pose a challenge to logistics on hauling, storage and conservation at the farm level. Cottonseed hulls and soybean hulls are also available in large quantities in Brazil. Therefore, wet distillers bran with solubles (WDBS) might be conserved combined with these drier ingredients to form a stable total mixed ration (TMR) silages or either partial mixed ration (PMR) silages to reduce conservation losses during storage and become more attractive to compose beef cattle finishing diets. When crop by-products are mixed with WDBS for silage, it can not only neutralize the disadvantage of excessive moisture but also improve the palatability of crop by-products (Yuan et al., 2015) as an associative effect. At the same time, through reducing effluents could reduce the losses of nutrients and environmental impact. The aim of this study was to identify strategies to control the spoilage and effluent losses in WDBS combined with dry ingredients for silage production.

Materials and methods

The experiment was designed into 5 treatments: TMR silage as positive control (50% WDBS, 7% tropical grass silage, 9% cottonseed hulls (CH), 12% soybean hulls (SH), 19% ground corn (CORN) and 3% minerals-vitamins premix); WDBS as negative control (WDBS exclusive); WDBS ensiled with cottonseed hulls (WDBS+CH, 85% WDBS+15% CH); WDBS ensiled with soybean hulls (WDBS+SH, 81% WDBS+19% SH); WDBS ensiled with ground corn (WDBS+CORN, 72% WDBS+28% CORN). CH, SH and CORN were mixed and ensiled according to the inclusion rate defined on the TMR silage treatment, which is based on a typical beef cattle finishing diet in Mato Grosso, Brazil. All treatments were mixed thoroughly and then packed into the 20 L polyethylene bucket silo during either 60 or 120 days with four replicates. The treatments were analyzed in a 5×2 factorial scheme (five treatments and two storage lengths). The statistical analysis was carried out using the MIXED procedure of SAS 9.4, and the model tested the effects of treatment, time and the interaction between these factors. Means were considered different when $p < 0.05$ by Tukey test.

Results

TMR treatment presented the highest lactic acid bacteria (LAB) counts, acetic acid and propionic acid concentration ($p < 0.01$), highest pH ($p < 0.01$), lowest dry matter (DM) losses ($p < 0.05$) and effluent production ($p < 0.05$) compared with other treatments. WDBS+SH had higher lactic acid concentration with decreased DM losses and effluents production among the other PMR silages. Prolonged length of storage increased pH, DM losses and effluents production. Fermentation and losses in silages, measured during the ensiling period and mixed either as TMR or PMR silages were dependent of the associative effects of the individual Water Retention Capacity (WRC) from single ingredients.

Conclusions

The results suggested that the strategy of mixed ensiled high moisture WDBS with other feedstuff was an effective method of preserving WDBS. All WDBS-based PMR silage were well-preserved with a low pH and high aerobic stability. WDBS+SH treatment reached the lowest dry matter loss and effluent net flow. WDBS-based TMR showed a relatively higher pH, however, it did not affect the fermentation quality and aerobic stability, moreover, showed lower dry matter loss and effluent production than WDBS- based PMR.

Table 1. Fermentative profile of TMR silage and PMR silage.

Items	Length of storage (d)	Treatments ¹						SEM ²	P-value ³		
		TMR	WDBS	WDBS+ CH	WDBS+ SH	WDBS+ CORN	Mean		T	L	T×L
pH	60	4.34Aa	3.73Bc	3.78Bbc	3.79Bb	3.74Bc	3.87				
	120	4.34Aa	3.87Ab	3.92Ab	3.92Ab	3.88Ab	3.99	0.01	<0.0001	<0.0001	<0.0001
	Mean	4.34	3.80	3.85	3.85	3.81	-				
Lactic acid, % DM corr	60	4.74	4.95	3.69	5.07	4.48	4.59B				
	120	5.49	6.08	6.14	8.64	7.51	6.77A	0.61	0.0269	<0.0001	0.1284
	Mean	5.12ab	5.52ab	4.91b	6.85a	5.99ab	-				
Acetic acid, % DM corr	60	0.37	0.30	0.24	0.30	0.31	0.30B				
	120	0.40	0.31	0.31	0.40	0.36	0.36A	0.03	0.003	0.0051	0.4657
	Mean	0.39a	0.31b	0.27b	0.35ab	0.33ab	-				
Propionic acid, mg/kg	60	383	140	130	134	120	181B				
	120	351	145	176	172	152	199A	17.9	<0.0001	0.0133	0.1672
	Mean	367a	143b	153b	153b	136b	-				
Butyric acid, mg/kg	60	117Aa	8.50Abc	3.25Ad	3.75Acd	12.5Ab	29.0				
	120	105Aa	11.0Ab	3.25Ac	6.00Abc	5.00Bc	26.1	3.22	<0.0001	0.6944	0.0176
	Mean	111	9.75	3.25	4.88	8.75	-				
Ethanol, % DM corr	60	0.11Aa	0.10Aa	0.10Aa	0.13Ba	0.11Ba	0.11				
	120	0.10Ab	0.12Ab	0.13Ab	0.20Aa	0.19Aa	0.15	0.01	<0.0001	<0.0001	0.0007
	Mean	0.10	0.11	0.11	0.17	0.15	-				

¹ TMR, Total mixed ration; WDBS, ensiling wet distillers bran plus solubles alone; WDBS+CH, mixed ensiled 85% WDBS with 15% cottonseed hulls; WDBS+SH, mixed ensiled 81% WDBS with 19% soybean hulls; WDBS+CORN, mixed ensiled 72% WDBS with 28% ground corn. ² SEM, Standard error of the mean. ³ T, Treatment effect; L, Length of storage effect; T×L, Interaction effect between treatment and length of storage

Values followed by different lowercase letters indicate statistical difference between treatments. Values followed by different capital letters indicate statistical difference between length of storage. Both differ statistically using the Tukey test at 5% probability.

Table.2. Effluents and dry matter losses of TMR silage and PMR silage.

Items	Length of storage (d)	Treatments ¹						SEM ²	P-value ³		
		TMR	WDBS	WDBS+ CH	WDBS+ SH	WDBS+ CORN	Mean		T	L	T×L
DM losses,%	60	1.80Ac	5.05Bb	7.85Aa	3.83Bbc	6.33Bab	4.97				
	120	0.58Ab	7.48Aa	7.65Aa	6.28Aa	8.75Aa	6.15	0.61	<0.0001	0.005	0.0089
	Mean	1.19	6.26	7.75	5.05	7.54	-				
Production of effluent (g/kg, FM) ⁴	60	26Ac	120Ba	101Aab	40Bc	86Bb	74				
	120	35Ad	180Aa	115Ab	57Ac	120Ab	101	5.30	<.0001	0.007	0.0002
	Mean	31	150	108	48	103	-				

¹ TMR, Total mixed ration; WDBS, ensiling wet distillers bran plus solubles alone; WDBS+CH, mixed ensiled 85% WDBS with 15% cottonseed hulls; WDBS+SH, mixed ensiled 81% WDBS with 19% soybean hulls; WDBS+CORN, mixed ensiled 72% WDBS with 28% ground corn. ² SEM, Standard error of the mean. ³ T, Treatment effect; L, Length of storage effect; T×L, Interaction effect between treatment and length of storage. ⁴ In fresh matter (FM).

Values followed by different lowercase letters indicate statistical difference between treatments. Values followed by different capital letters indicate statistical difference between length of storage. Both differ statistically using the Tukey test at 5% probability.

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Essential Oil Compounds as Additives for Rehydrated Corn Grain Silage

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Keywords: aerobic stability, essential oils, fermentation, rehydrated corn grain silage.

Introduction

Essential oils have been experimented with as additives for forage silages with promising results such as enhanced aerobic stability and improvements in conservation quality. However, published trials are still limited to crude oils extracted from plants, and there is a lack of information regarding the use of essential oils as additives for grain silages. This study aimed to identify molecular compounds isolated from essential oils that could be promising as strategic additives for rehydrated corn grain silage (RCGS).

Materials and methods

Silages were made by rehydrating dry ground corn grains to 350 g/kg of moisture and packing ($>1,000 \text{ kg/m}^3$) in 5-L plastic buckets. Treatments were prepared as a factorial arrangement of 2 storage times (30 and 60 d) $\times$ fifteen strategies, being: control RCGS (CT); carvacrol at 200 (CAL), 600 (CAM) and 1,000 (CAH) mg/kg fresh matter (FM); cinnamaldehyde at 200 (CIL), 600 (CIM) and 1,000 (CIH) mg/kg FM; D-limonene at 200 (LIL), 600 (LIM) and 1,000 (LIH) mg/kg FM; citral at 200 (CRL), 600 (CRM) and 1,000 (CRH) mg/kg FM; commercial microbial inoculant (MI) containing *Lentilactobacillus buchneri* ($1.12 \times 10^5 \text{ cfu/g FM}$), *Lactiplantibacillus plantarum* ($9.6 \times 10^4 \text{ cfu/g FM}$) and *Propionibacterium acidipropionici* ($1.12 \times 10^5 \text{ cfu/g FM}$); and sodium benzoate at 2 g/kg FM (BZ). The MI and BZ strategies were considered positive controls. The RCGS were evaluated for dry matter (DM) losses, microbial and fermentative profile, and aerobic stability.

Results

The treatment of RCGS with sodium benzoate at 2 g/kg FM promoted higher quality of conservation, decreasing DM losses in the silo (Figure 1), and suppressing populations of yeasts and molds, which also markedly enhanced the aerobic stability (Figure 2). The treatment of RCGS with a microbial inoculant combining *L. buchneri*, *L. plantarum* and *P. acidipropionici* did not yield the expected results though, failing in achieve better outcomes for conservation and aerobic stability. Thus, we chose the BZ strategy as the positive control treatment to discuss the results for the strategies based on essential oil compounds in this study. Carvacrol decreased lactic acid production, especially at the higher doses (600 and 1,000 mg/kg FM), which compromised the acidification of RCGS, with CAM and CAH presenting the highest pH values in our study (4.1 and 4.2, respectively). The hampered acidification probably favored undesirable fermentations, as greater DM losses were observed (Figure 1) and even a favoring of *Clostridium* development occurred on the CAH silage, which presented an exceptionally high concentration of butyric acid (4,500 mg/kg DM). However, carvacrol showed promising results for aerobic stability, with CAH at 60 d of storage being able to emulate the effects of BZ (Figure 2). The CIH strategy was effective at inhibiting yeasts and was the only strategy besides BZ capable of suppressing fungi. Both CIM and CIH were able to decrease ethanol content. Curiously, the extended storage time from 30 to 60 d reduced the efficiency of CIH in suppressing yeasts, accompanied by a decrease in lactic acid content. It seems that storage time may be an important factor regulating the efficiency of cinnamaldehyde as an antimicrobial agent in grain silage. All strategies with cinnamaldehyde promoted lower pH values for RCGS at both storage times (ranging from 3.9 to 4.0), which probably enhanced the quality of conservation of the silages, as CIM and CIH were able to emulate the decreased DM losses observed for the BZ strategy (Figure 1). Only CIH managed to enhance aerobic stability of RCGS, although it was not able to emulate BZ (Figure 2). D-limonene did not prevent DM losses nor consistently improved aerobic stability with doses varying from 200 to 1,000 mg/kg FM. Citral also did not improve silage conservation quality nor its aerobic stability. Both compounds were deemed not promising as additive for RCGS.

Conclusions

Of the compounds tested in this study, only carvacrol and cinnamaldehyde proved promising as strategic additives for RCGS. Carvacrol enhanced aerobic stability with increasing doses, being able to emulate sodium benzoate when applied at 1,000 mg/kg FM in RCGS stored for 60 d, although this enhancement may be associated to the increased butyric acid production. Carvacrol, however, compromised the acidification and increased DM losses in the silo. Cinnamaldehyde decreased DM losses, being able to emulate sodium benzoate when applied at doses of 600 and 1,000 mg/kg FM. However, even the enhanced aerobic stability with the 1,000 mg/kg FM dose was not able to emulate sodium benzoate, and higher doses will be experimented in a future trial. D-limonene and citral did not promote consistent results desirable for RCGS within the doses and storage times evaluated in this study.

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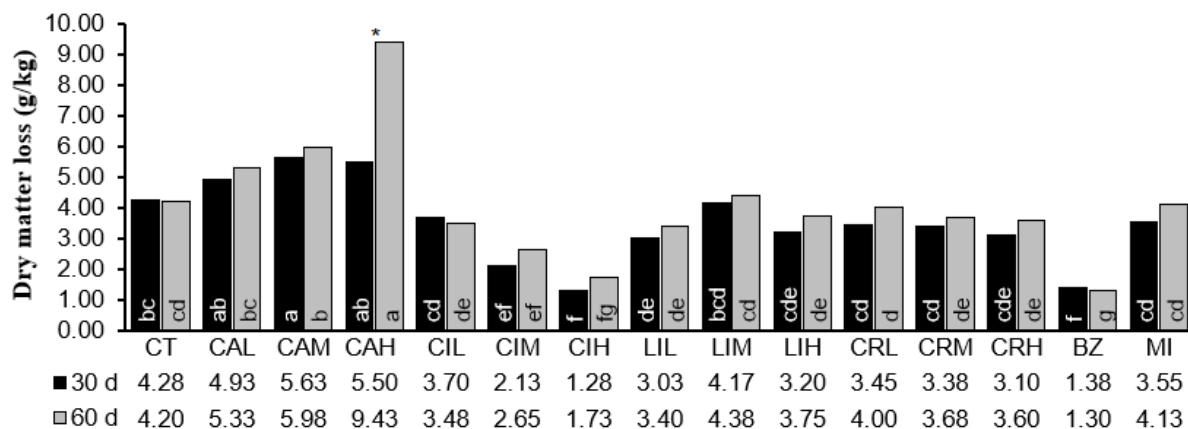


Figure 1. DM loss of RCGS according to the treatments. Effect of strategy, $P < 0.01$; effect of storage time, $P < 0.01$; interaction strategy $\times$ storage time, $P < 0.01$. SEM = 0.15. Means with different letters differ by Tukey test ($P < 0.05$) within storage time (bars of same color). (*) Effect of storage time ($P < 0.05$) within the strategy.

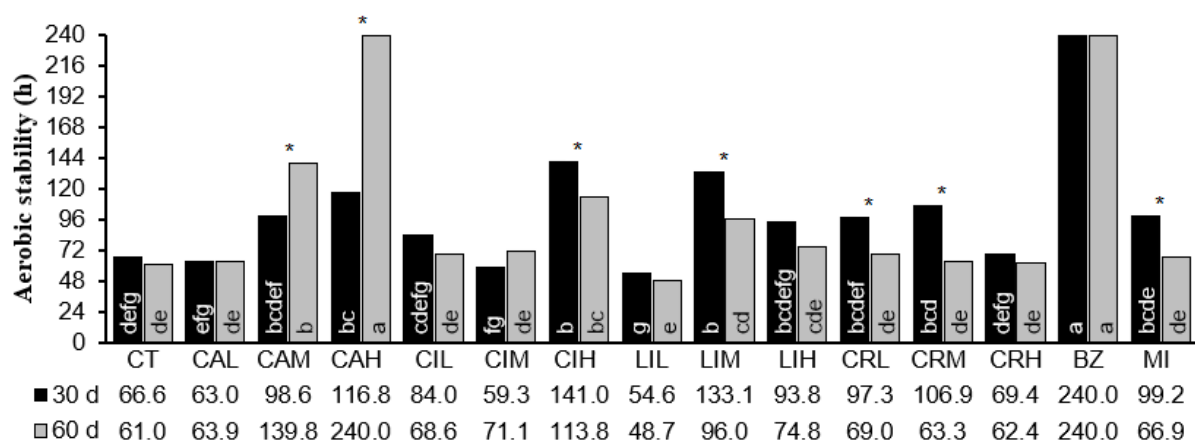


Figure 2. Aerobic stability of RCGS according to the treatments. Effect of strategy, $P < 0.01$; effect of storage time, $P = 0.32$; interaction strategy $\times$ storage time, $P < 0.01$. SEM = 6.5. Means with different letters differ by Tukey test ($P < 0.05$) within storage time (bars of same color). (*) Effect of storage time ($P < 0.05$) within the strategy.

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Propionic Acid-based Additive on Aerobic Stability of Total Mixed Ration – I: Dairy Cows Performance and Feed Frequency

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Keywords: aerobic stability, dairy cows, feeding frequency, total mixed ration.

Introduction

Tropical climates present environmental challenges to the stability of total mixed ration (TMR) in the feed bunk throughout the day. Increasing feeding frequency is a typical suggested strategy to compensate for TMR deterioration and to prevent refusal by animals. However, treatment with a propionic acid-based additive could stabilize the TMR enough to allow a reduction in daily feeding frequency.

Materials and methods

A preliminary trial was performed to select the most effective dose of the additive (Fresh CutTM Plus, Kemin South America, Brazil) based on the monitoring of the aerobic stability of TMR. Data was analyzed as a completely randomized design including fixed effect of dose, and means were compared by polynomial regression ($p \leq 0.05$). The additive was effective on improving aerobic stability of TMR compared to the control treatment. However, there was no difference across doses starting from 2 mL/kg fresh matter (FM) (Table 1). Our results suggested the dose of 2 mL/kg FM as the most effective for improvement of the aerobic stability of TMR, and this dose was selected for the Additive strategy in the animal performance trial. Twelve Holstein cows (mean $\pm$ SD; 612 $\pm$ 158 kg of body weight, 27 $\pm$ 6.5 kg/d milk yield, 178 $\pm$ 60 days in milk, and 3.0 $\pm$ 0.5 of body condition score) were assigned to a switch-back design to evaluate the effect of two feeding management strategies: Control, with two daily feeding of a control TMR; and Additive, with cows receiving a single daily feeding of a TMR treated with the additive at 2 L/t FM. The TMR was composed of 44.59% corn silage, 6.40% Tifton wilted silage, 21.11% rehydrated corn grain silage, 9.25% citrus pulp, 16.33% soybean meal and 2.32% mineral-vitamin mix. The animals were housed in a free-stall barn equipped with individual feed bunks, capable of individual access control and feeding behavior monitoring system (Intergado Ltda., Contagem, Minas Gerais, Brazil). The cows were subjected to two daily milkings, at 5 am and 5 pm. At the afternoon feed delivery for the Control diet, the TMR was manually mixed in the feed bunks for both treatments throughout three minutes, immediately before the animals had access to it, in order to stimulate consumption randomly. The parameters of animal performance included fixed effect of treatment, random effect of period (1, 2, 3), and random effect of animal. All statistical analyses were made using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Cows fed the Additive strategy significantly increased daily milk yield by 10.2% without changes in dry matter intake, although no difference was observed for the feed efficiency (Table 2). Milk fat content was decreased for cows in the Additive strategy. An increase in milk somatic cell counts was observed for cows in the Additive, which could be related to the faster first meal activity after milking than the cows in the Control strategy, rendering them more susceptible to teat contamination. Milk energy and milk urea nitrogen contents were not affected by treatments, although energy excretion through milk was greater for the cows in the Additive. The Additive strategy promoted a shift in the sorting behavior in favor of longer particles, increasing the time spent on rumination, and decreased the number of daily meals at the expense of larger meal size.

Conclusions

The treatment of TMR with the propionic acid-based additive improved its aerobic stability and enabled the strategy of a single daily feeding to dairy cows with an increased milk yield by 10.2% and rumination time by 9.7%. The additive-treated TMR promoted a shift in the sorting behavior in favor of longer particles, increasing the time spent on rumination, and decreased the number of daily meals (31.5%) at the expense of larger meal size (21.4%), without changes in dry matter intake. Cows fed the Additive TMR also spent less time with first meal post-milking, resulting in higher SCC.

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Table 1. Preliminary aerobic stability trial of total mixed ration.

Item	Additive dose (/kg FM)						SEM	L	Q	D
	0 mL	1 mL	2 mL	3 mL	4 mL	5 mL				
Aerobic stability, h	47.8	53.0	73.8	73.5	74.1	75.4	3.01	<0.01	<0.01	0.11
T_{max}, °C	41.2	39.4	38.2	38.3	38.2	27.0	0.00	<0.01	<0.01	<0.01
TT_{max}, h	170.0	169.3	169.1	168.8	169.0	145.8	2.28	<0.01	<0.01	<0.01

T_{max} – maximum temperature; TT_{max} – time to achieve maximum temperature.

Table 2. Performance of dairy cows fed the experimental total mixed rations.

Item	Treatment		SEM	P-value
	Additive	Control		
Dry matter intake, kg/d	22.3	21.6	0.89	0.59
Milk, kg/d	25.9	23.5	1.34	0.01
Feed efficiency	1.22	1.15	0.08	0.42
Energy-corrected milk, kg/d	25.1	23.2	1.16	0.03
Fat, %	3.28	3.41	0.07	0.01
Crude Protein, %	3.26	3.26	0.06	0.92
Lactose, %	4.44	4.48	0.03	0.06
Milk NE_L, Mcal/kg	0.66	0.67	0.01	0.12
NE_L, Mcal/d	16.8	15.6	0.77	0.04
Somatic cell count, mil/mL	99.3	41.8	8.10	<0.01
Milk urea nitrogen, mg/dL	12.5	13.1	0.27	0.27
Particle sorting index, % as fed				
> 19 mm	114.3	84.2	3.53	<0.01
8-19 mm	97.2	101.4	0.66	<0.01
4-8 mm	98.3	98.8	0.72	0.76
< 4 mm	98.6	102.4	0.73	<0.01
Ingesting, min/d	188	258	9.2	<0.01
Ruminating, min/d	599	546	10.1	<0.01
Idleness, min/d	625	621	12.6	0.82
Water intake, min/d	27	21	2.1	0.05
Meal frequency, meals/d	6.1	8.9	0.27	<0.01
Meal size, kg	3.4	2.8	0.19	0.03
Meal length, min	20.0	20.8	1.00	0.49
1st meal (morning feeding), kg	4.4	4.4	0.32	0.84
1st meal (morning feeding), min	21.7	32.3	2.21	<0.01
1st meal (afternoon feeding), kg	3.7	3.4	0.29	0.42
1st meal (afternoon feeding), min	20.3	28.0	2.02	<0.01

Bacterial Dynamics in Mixtures Based on Forage Cactus and Sorghum Silage, with or Without Fecal Contamination, During Aerobic Exposure

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Keywords: animal production, *Escherichia coli*, metataxonomy, pathogen

Introduction

The forage cactus (*Nopalea cochenillifera* Salm Dyck) is a cactaceous plant of great importance in Arid and Semi-Arid regions, characterized by low dry matter (DM) content and high levels of non-fibrous carbohydrates (NFC), making it a vital energy source for ruminants. Additionally, the forage cactus has low concentrations of neutral detergent fiber (NDF) and crude protein (CP) in its chemical composition. This can result in nutritional disturbances in ruminants when used in high proportions or as a sole feed source, leading to soft stools (Pereira et al., 2021).

However, recent studies have reported that digestive problems associated with high proportions of forage cactus in ruminant diets may also be linked to contamination by potentially pathogenic microorganisms present during feed preparation and provision (Sato et al., 2016; Paulino et al., 2021). Therefore, post-harvest handling could predispose pathogenic microorganisms, leading to nutritional disturbances in ruminants fed with high proportions of forage cactus or when it is used as the sole forage source in the diet.

Given this, other feedstuffs can be associated with forage cactus to inhibit the growth of these pathogenic microorganisms, such as forage sorghum silage (*Sorghum bicolor* L. Moench). The use of this conserved forage source is a common practice, as the ensiling process aims to preserve the nutrients in the plant, allowing its storage and use during periods of pasture scarcity. Moreover, this plant is characterized by homolactic fermentation, with pH stabilizing between 3.8 and 4.2 (Dos Santos et al., 2023; Lucena et al., 2023). Thus, we hypothesize that the pH of the ensiled mass inhibits the growth of potentially pathogenic microorganisms in diets containing high proportions of forage cactus, thereby improving its hygienic quality. Accordingly, the present study aims to evaluate the growth of potentially pathogenic microorganisms in mixtures of forage sorghum silage and fresh forage cactus, with or without fecal contamination, depending on the period of aerobic exposure.

Materials and methods

The experiment was conducted at the Forage Laboratory of the Center for Agricultural Sciences (CCA) at the Federal University of Paraíba (UFPB), Campus II, located in the municipality of Areia, Paraíba, Brazil. The experimental design used was completely randomized in a $2 \times 2 \times 2$ factorial scheme, with two proportions of forage cactus (80% and 20%, based on fresh matter), with or without fecal contamination, and two aerobic exposure times (0 and 24 hours), based on fresh matter, with four replicates. The forage sorghum silage used in the experiment exhibited the following fermentation profile: pH of 3.9; lactic acid concentration of 72.80 g/kg DM; acetic acid, 35.68 g/kg DM; propionic acid, 14.10 g/kg DM; and butyric acid, 0.017 g/kg DM. The ammoniacal nitrogen concentration was 16.29 g of N-NH₃ per kg of total nitrogen (TN).

For genomic DNA extraction, 25 g of each sample was diluted in 225 mL of sterile 0.85% saline solution, homogenized for 3 minutes, and filtered. The filtered solution was centrifuged at 6,000 rpm for 10 minutes at 4°C, preserving the pellet and discarding the supernatant. DNA was extracted using the Powersoil Pro DNA Kit (Qiagen, Hilden, Germany), evaluated by 1% agarose gel electrophoresis, and quantified by fluorometry (Qubit, ThermoFisher, Waltham, MA, USA). The V3-V4 regions of the 16S rRNA gene were amplified by PCR. The amplicon library was prepared using the Nextera XT Index Kit Set A (Illumina Inc., San Diego, CA, USA) and purified with magnetic beads (Agencourt AMPureXP, Beckman Coulter, Indianapolis, USA). Paired-end sequencing was performed on the Illumina MiSeq platform with a 500-cycle V2 kit (Illumina Inc., San Diego, CA, USA). Reads were processed on the Qiime2 platform, where forward and reverse sequences were imported, and low-quality reads (Q>20) were removed using the Dada2 program.

For statistical analysis, the PERMANOVA test was used to evaluate alpha and beta diversity, respectively.

Results

It was possible to identify 461 and 1267 ASVs after the normalization of 19,200 reads in the samples containing 80% and 20% of forage cactus, respectively. There was no statistical significance regarding fecal contamination in the mixture with 80% forage cactus, while the contaminated mixture with 20% forage cactus showed higher diversity compared to the control group at 0 hours in the alpha diversity assessment.

Richness and evenness values were higher in the mixture with 80% forage cactus and lower in the mixture with 20% forage cactus at 24 hours, with no significance regarding contamination. The main phyla identified in the relative abundance analysis of the mixtures with 80% forage cactus were *Bacillota*, *Pseudomonadota*, and *Bacteroidota*. At both 0 hours and 24 hours, *Pseudomonadota* and *Bacillota* were predominant, with the additional presence of *Bacteroidota*. In the mixture with 20% forage cactus, *Bacillota* was more prevalent at 0 hours, while *Pseudomonadota* dominated after 24 hours of exposure.

At the family level, the analysis revealed that in the mixture with 80% forage cactus at 0 hours, there was a higher presence of *Lactobacillaceae* and *Acetobacteraceae*, and after 24 hours of exposure, there was a significant abundance of gram-negative bacteria, including *Clostridiaceae*. In the mixture with 20% forage cactus with fecal contamination at 0 hours, a higher presence of *Lactobacillaceae* and *Lachnospiraceae* was observed. However, at 24 hours, *Acetobacteraceae* dominated the mixture environment, with a reduced presence of *Lactobacillaceae*. The main genera detected at 0 hours in the mixture with 80% forage cactus were *Weissella*, *Acetobacter*, *Gluconobacter_A*, and *Lactiplantibacillus*. In the mixture with 20% forage cactus, a higher population of *Acetobacter* and *Gluconobacter* was identified at 24 hours of aerobic exposure. The presence of acetic acid bacteria in the silage with a higher proportion of forage sorghum (20% forage cactus) can be explained by the presence of ethanol, produced by yeasts in aerobic conditions.

Conclusions

The mixture with 80% forage cactus showed lower microbial diversity compared to the mixture with 20% forage cactus. However, the higher proportion of forage cactus was associated with higher-risk microbial groups, such as *Clostridiaceae*. On the other hand, the mixture with 20% forage cactus exhibited a greater presence of acetic acid bacteria. Therefore, based on the hygienic quality of the mixtures, the use of the mixture with 20% forage cactus is recommended.

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Contamination by *Escherichia coli* in Total Mixed Rations with a High Proportion of Forage Cactus

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Keywords: animal production, buffel grass hay, microbial population, ruminants.

Introduction

Forage cactus (*Nopalea cochenillifera* Salm Dyck) is a cactaceous plant of great importance in arid and semi-arid regions, characterized by its low dry matter content and high non-fibrous carbohydrate content, which makes it a source of water and energy for ruminants (Pereira et al., 2025). In addition, forage cactus has low concentrations of neutral detergent fiber, which can result in disorders in the gastrointestinal tract in ruminants when used in high proportions or as the sole source of forage in the diet (Sá et al. 2023).

The digestive problems associated with high proportions of forage cactus in ruminant diets can also be linked to contamination by potentially pathogenic microorganisms present during the mixing of ingredients and delivery of the diet (Paulino et al., 2021). In view of this, the feed management adopted on the farm has a direct influence on the final hygienic quality of the total mixed ratios (TMR) used to feed the animals. Santos et al. (2020), evaluating TMR silage based on forage cactus and gliricídia, observed a lower microbial diversity in the ensiled mass, with a greater predominance of bacteria from the *Bacillus* genus. Furthermore, the authors did not observe counts of undesirable microorganisms such as enterobacteria in the ensiled mass. Thus, we hypothesize that the fermentation process inhibits the growth of potentially pathogenic microorganisms, such as *Escherichia coli* (*E. coli*), in diets with high proportions of forage cactus. The aim of this study is to quantify the *E. coli* population in TMR containing high proportions of forage cactus and subjected to different feeding methods: *in natura* and silage.

Materials and methods

The experiment was conducted at the Forage Laboratory of the Center for Agricultural Sciences (CCA) at the Federal University of Paraíba (UFPB), Campus II, located in the municipality of Areia, Paraíba, Brazil. The experimental design used was entirely randomized, in a 2 x 2 x 2 factorial scheme, with two levels of buffel grass (*Cenchrus Ciliaris* L.) hay (7.5% and 45%), with and without faecal contamination, two types of feed management, TMR *in natura* and TMR silage, with a repeated measure in time (0, 6, 12, 24 hours of exposure to the environment), and four repetitions. At the time of opening, the TMR silages showed an average pH value of 4.7.

Fecal samples were collected directly from goat pens to simulate the contamination of TMR with *E. coli*. Then, 10 g of fresh feces were weighed and diluted in 90 mL of sterile distilled water. After dilution, the mixture was homogenized and filtered through sterile gauze to obtain an aqueous extract free of solid particles. This extract was used as an inoculum for the controlled contamination of the TMR.

Plating to quantify the *E. coli* population of the samples was carried out according to the methodology proposed by González and Rodríguez (2003). The EMB Levine Eosin Blue Methylene Agar (Kasvi, K25 - 1050, Brazil, 2022) is a selective culture medium that inhibits the growth of gram-positive bacteria and indicates whether the bacteria are lactose fermenters, where the *E. coli* colonies were easily identifiable by their metallic green color. Only plates containing between 30 and 300 CFU were considered for counting. Tukey's test at 5% probability was used for statistical analysis.

Results

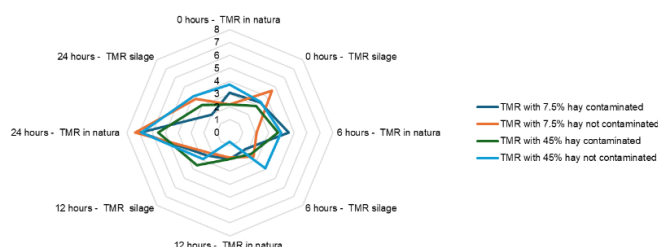


Figure 1. *Escherichia coli* population (Log CFU/g) in a total mixed ratios with high proportions of forage cactus, containing different levels of buffel grass hay (7.5 and 45%), contaminated and non-contaminated, evaluated over the time of exposure to the environment (0, 6, 12 and 24 hours) in two forms of feed management: *in natura* and silage.

It was observed that, regardless of the hay content used (7.5 and 45%) in the mixtures and the contamination, the TMR *in natura* showed an increase in the *E. coli* population after 24 hours of exposure to the environment, reaching values of over 6.85 Log CFU/g. On the other hand, TMR silage maintained lower counts over time, indicating a positive effect of the ensiling process in reducing the *E. coli* population.

The 7.5% hay content in TMR *in natura* resulted in a rapid multiplication of *E. coli* in the first 6 hours, with an increase of approximately 1.35 Log CFU/g compared to time 0. However, there was a reduction at 12 hours of exposure, but it increased considerably again after 24 hours (6.92 Log CFU/g). TMR silage, on the other hand, even with a similar initial value (3.32 Log CFU/g), showed a reduction in population over time, with a population of 1.97 Log CFU/g after 24 hours. For the 7.5% hay content in the uncontaminated TMR *in natura*, a similar result was observed, with the *E. coli* population increasing after 24 hours of exposure to the environment (7.35 Log CFU/g), while the TMR silage maintained the lowest population over time (3.70 Log CFU/g).

The same trend was observed for the *E. coli* population counts in the 45% hay content in the contaminated TMR *in natura*, where the population at time 0 was 2.18 Log CFU/g, increasing considerably after 24 hours of exposure to the environment (5.57 Log CFU/g). TMR silage, on the other hand, maintained counts of less than 3.03 Log CFU/g. A similar result was observed for the 45% hay content in uncontaminated TMR, with higher populations in fresh TMR and lower in silage TMR, reinforcing the beneficial effect of the ensiling process on the hygienic quality of TMR.

Conclusions

The ensiling process plays an important role in reducing microorganisms with pathogenic potential such as *E. coli* in TMR, helping to maintain its hygienic quality. In addition, contamination influences the *E. coli* population, reinforcing the importance of hygiene during the mixing and feeding process. Based on the results obtained, the adoption of TMR silage with 7.5% hay is recommended as the most effective feeding strategy for reducing gastrointestinal tract disorders in ruminants.

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Formic Acid Recovery Analysis as a Farm Advisory Tool

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Keywords: ensiling, silage additive application, losses, effluent, volatilization.

Introduction

In Northern Europe, formic acid is widely used to preserve wet, low-sugar forages by inhibiting undesirable fermentation and reducing nutrient losses. However, variation in silage fermentation quality could result from inadequate application of additives. The objective was to evaluate formic acid recovery as a diagnostic tool for farm advisory purposes, aiming to identify and describe factors influencing recovery rates to give recommendations for improved silage management.

Materials and methods

A meta-analysis was conducted using data from 172 silages from ensiling trials at Natural Resources Institute Finland (Luke, laboratory accredited SFS-EN ISO/IEC 17025, FINAS T024) between 2010 and 2023. The dataset comprised 32 corn silages ensiled at four dry matter (DM) levels (23, 26, 28 and 36 %), 106 timothy-meadow fescue (TM) silages ensiled at six different DM levels (15, 22, 24, 31, 36 and 54 %) and 34 pea-oat-wheat-whole crop (WC) silages with a DM of 22 %. Of these, 11 silages were untreated, while 161 were treated with 18 different formic acid-based additives. Effluent production was recorded for 23 low DM silages. Silo types included plastic vacuum bags (38 silages) and cylinder silos (134 silages). Details of the methods are available in Franco et al. (2023). Formic acid concentrations were analyzed via a water extraction method (1:15 dilution) and quantified through a commercial kit (r-Biopharm UV-method, Art. No.: 10979732035, manufacturer Roche Diagnostics) using UV-VIS double-beam UV-1800 spectrophotometer (Shimadzu Co., Kyoto, Japan).

Results

The analysis identified effluent loss and evaporation as primary factors influencing formic acid recovery. Formic acid recovery from silages that excreted effluent averaged 70% from TM15 (range 59–77%) and from WC22 46% (range 20–75%). No effluent was lost from bag silages. However, in WC22 silages ensiled in bags, formic acid recovery was only 44%. Those silages had a pH around 4, at which 36% formic acid remains undissociated, enabling evaporation and exit from the gas-permeable bag. Continuously more formic acid was converting to undissociated form to maintain the pH-dependent equilibrium. This process is likely to explain the loss of formic acid from bag silages. Even when dry matter was higher (TM36), formic acid recovery was lower in bag silages compared to cylinder silo silages (83% vs. 90%). In contrast, prewilted silages ensiled in cylinder silos demonstrated higher recovery rates, averaging 92% (standard deviation 10%; 107 silages). The regression analysis resulted in an intercept close to zero (Figure 1), data included four data points from untreated silages (111 silages). Notably, there was no correlation between formic acid recovery rates and silage DM content (Figure 2).

Formic acid-based silage additives play a critical role in preserving forage quality and preventing nutrient losses. Those additives restrict fermentation, prevent proteolysis and clostridial fermentation and minimize losses (Seppälä et al., 2016). However, challenges such as underdosing and uneven application can result in suboptimal fermentation, adversely impacting livestock performance. Such failures can lead to reduced silage intake, deficiency in fiber and energy availability, and ultimately decreased milk yield and health issues for high-producing dairy cows. Poor silage fermentation quality is often an unexpected occurrence that requires immediate changes in feeding management, along with advisory measures to prevent similar issues in the future. Analyzing formic acid recovery can be a valuable tool for identifying the potential causes for poor silage quality. Proper sampling, packing and storage practices are essential to prevent evaporation losses of formic acid from silage samples prior to analysis. Effluent loss must also be accounted for when interpreting recovery results, particularly in wet silages. When underdosing of the additive is suspected, application practices should be reviewed. Weighing the grass loads is highly recommended to ensure proper application levels. Furthermore, it is essential to check that spraying nozzles are correctly positioned to minimize evaporation losses (Nysand et al., 2012).

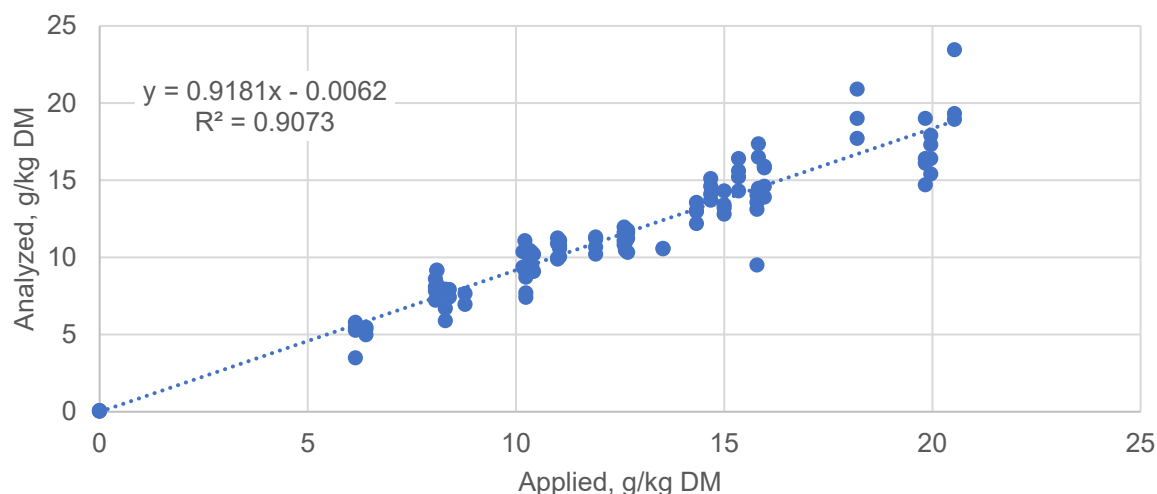


Figure 1. Formic acid recovery from 111 prewilted silages ensiled in cylinder silos.

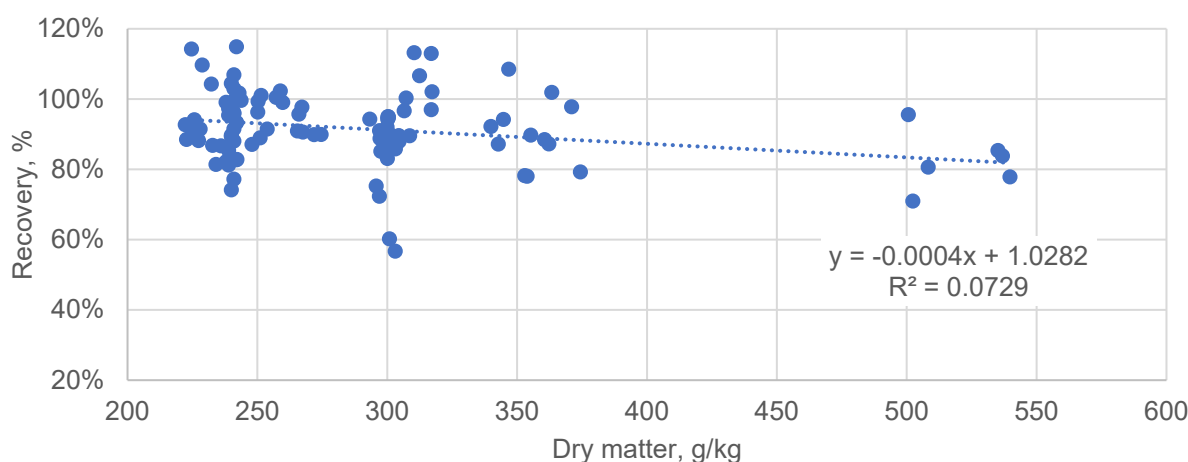


Figure 2. Relationship of formic acid recovery with sample dry matter concentration.

Conclusions

Formic acid analysis is a valuable tool for supporting advisory work on farms, verifying the accuracy of additive application, and encouraging farms to weigh loads to ensure the correct application rate of silage additives. Throughout the entire process, from silage sampling to analysis, it is essential to handle samples in a way that minimizes potential evaporation losses of formic acid. Additionally, when interpreting the results of formic acid analysis of wet silages, it is important to consider that potential losses in effluent can reduce recovery rates.

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A Review (2013-2022) of Strategies for Adapting Silage Production Systems to Climate Change in Dry Continental Regions of Central Europe

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Keywords: Climate change, early-cut whole-crop rye silage, intensive grass silage

Introduction

Since the 1970s, corn silage and alfalfa silage have been the two main forage components of the dairy cow diet in dry continental region of Central Europe. High variability in yield and nutritive values during the last 10 years show that the corn silage is at high risk in the dry continental region of Central Europe (Orosz and Balogh, 2023). Detection of *Aspergillus* spp. infections and aflatoxinB1 contamination on the field in corn plant after dry and hot seasons is another aspect of the challenge. Therefore, strategic crop rotation decisions were needed to increase the safety of forage supply and milk production in the dairy sector on dry and hot lands of Central Europe. There are options of stabilizing the forage supply before (or beside) the installation of irrigation. Winter crops provide more advantages. Intensively grown (on arable), annual, high sugar grasses (*Lolium multiflorum*, Festulolium-type grass hybrids) and whole-crop winter cereals such as winter rye (*Secale cereale*) cut in early spring are not exposed to the potentially hot and dry periods of the year (Auerbach and Theobald, 2020). Double cropping is another opportunity. Due to early spring harvest, the dual-crop combinations are reliable solutions for dry continental regions: winter whole-crop rye and corn, high sugar grass and BMR sorghum can increase total biomass yield/year and have environmental benefits, too (Ketterings et al, 2015). The author shows the 10 years database (nutritive values and digestibility) of whole-crop early cut rye silages (*Secale cereale*) and intensively grown, annual, high sugar grass silages (*Lolium multiflorum*, Festulolium-type grass hybrids).

Materials and methods

Samples derived from large scale farms (average farm size: 410 dairy cows/farm in 2023) in Hungary between 2013-2022 (whole-crop rye silage cut before heading, n=1379; intensive grass silage n= 910). Results based on routine laboratory NIR analyses (Livestock Performance Testing Ltd, Hungary). Samples were dried at 70°C and ground according to the Guidelines of SamplinQ® system (Eurofines Agro, Wageningen, The Netherlands, L54 2009/152). Spectra were determined according to the guidelines of NEN-EN-ISO 12099 (Q-Interline Quant FT-NIR analyser, ISO 12099:2010 guidelines for the application of near infrared spectrometry). Measured parameters are given in Table 1-2.

Results

As early-cut rye silage and intensive grass silage have already proven their benefits in practice and have recently become accepted and widespread in the dairy sector, we highlight only the challenges that need to be improved. The average crude protein content (Table 1) of the early cut rye silages and grass silages is an extreme weakness of our technology. The goal is to reach at least 150-160 g/kg DM in the future in order to increase the ratio of the home-grown protein in the diet. While we are also tasked with further reducing nitrate levels in whole-crop rye silages and intensive grass silages. The average ash contents (Table 1) of early-cut rye and intensive grass silages were not ideal, giving indication for the need of better field management control. The 48-hour fiber digestibility were 65%NDF and 66%NDF (Table 2), which are far above the average of corn silage (2023: 61±1.6%NDF, n=414) and alfalfa silage values (40±7.1%NDF, n= 3043). Improving this parameter above 70% is a challenge for the future. The average digestible fiber content of rye silage (cut before heading) was higher than that of intensive grass silages. Otherwise, the digestible fiber content of both silages was more than twice that of alfalfa silages (2013-2022 alfalfa silage: 166 g/kg DM dNDF₄₈, n=3043) and 50-60% higher than corn silage (2020 corn silage: 218 g/kg DM dNDF₄₈, n=411).

Table 1 Nutrient content of early cut whole-crop rye silage (n= 1379) and early cut intensive grass silage (n=910) within the period of 2013-2022 based on 1000 g dry matter (NIR database, LPT Ltd.)

Type of forage	Dry matter	Crude protein	Crude fat	Crude fiber	Ash	Total sugar	Nitrate	Soluble protein
Early cut whole-crop rye silage								
Mean (g)	291	137	35	291	106	39	3.5	111
SD	65.8	29.6	5.0	45.5	34.0	30.5	2.66	25.7
BIAS1	-0.53	-0.29	-0.09	-0.88	-	-0.59	0.23	-
R2 2	0.97	0.99	0.89	0.98	-	0.97	0.81	-
Intensive, early cut grass silage								
Mean (g)	340	143	34	271	116	59	4.3	95
SD	82.5	31.7	6.0	34.9	25.8	40.9	3.4	23.4
BIAS1	-0.41	0.25	-0.09	-2.47	-	1.02	0.14	-
R2 2	0.96	0.98	0.91	0.93	-	0.96	0.85	-

¹BIAS- the average difference between predicted and actual value, ²R² –coefficient of determination.

Table 2 Fiber fractions, organic matter and fiber digestibility of early cut whole-crop rye silage (n= 1379) and early cut intensive grass silage (n=910) within the period of 2013-2022 based on 1000 g dry matter (NIR database, LPT Ltd.)

	aNDFom ¹	ADL	NDFd ₃₀ ²	NDFd ₄₈ ²	dNDF ₄₈ ³	OMd ₄₈ ⁴
	g	g	%NDF	%NDF	g	%
Early cut whole-crop rye silage						
Mean	544	26	53	66	353	71.6
SD	72.0	8.1	6.5	6.5	39.3	4.7
BIAS ⁵	1.26	0.11	0.38	-	-0.31	-
R ² 6	0.97	0.97	0.94	-	0.93	-
Intensive early cut grass silage						
Mean	499	25	54	65	322	73
SD	58.9	8.2	8.1	7.7	35.3	5.3
BIAS ⁵	-2,31	-0.31	-0,13	-	-1.12	-
R ² 6	0,94	0,93	0.80	-	0.95	-

¹aNDFom₄₈ - amylase treated and ash corrected NDF rumen degradability (48 hours *in vitro* incubation- NIR data), ²⁻³NDFd_{30, 48} - amylase treated and ash corrected NDF rumen degradability (30 or 48 hours *in vitro* incubation- NIR data), ⁴OMd₄₈ - organic matter digestibility (48 hours *in vitro* incubation- NIR data). ⁵BIAS- the average difference between predicted and actual value, ⁶R² –coefficient of determination

Conclusions

We can conclude that there are still challenges in cultivation and harvesting technology of early-cut rye silage and intensive grass silages. However, even the non-optimal quality is an improvement over corn silage- and alfalfa silage-based diet from fiber digestibility and digestible fiber content point of view in the high milking dairy cow diet.

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Well Digestible Forages for Feeding During Heat Stress (Dataset 2013-2022)

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Keywords: Heat stress, fiber digestibility, indigestible fiber

Introduction

For lactating dairy cows in dry continental region, the most negative dietary factor during the summer period is usually the low dry matter- and energy intake. The higher number of heat stress days due to climate change in dry continental low lands (below 500m altitude) of Central Europe (Tóthi et al., 2024) increases the animal health risk. However, dry matter intake, feeding and rumination behaviour, efficiency of milk production can be influenced by digestion features of NDF (Grant and Cotanch, 2012). There is a well-known correlation between the forage's NDF digestibility and dairy cow milk production: the expectable effect is 0.18 kg d⁻¹ more dry matter intake (DMI) and 0.25 kg d⁻¹ more 4% fat-corrected milk for every 1% increase of NDF digestibility (Oba and Allen, 1999). Well digestible forages flow through the rumen more quickly (fast pool), increasing dry matter intake, while reducing relative heat increment in the rumen. When dairy cows consume high-quality and well digestible forage, it may lead to a higher economic return, also, due to less grain consumption and higher income-over-feed-cost (Chase, 2012).

Cereals were grown for their grains in the past, but nowadays there are other options in dairy cow nutrition. The soft dough stage is still recommended in areas with too cool growing seasons for corn to provide missing starch in the diet. Recently, in dry continental regions (below 500 m altitude) whole-crop cereals are recommended to harvest at the flag-leaf stage to prevent the lack of digestible fiber in high milking dairy cow diet (Tóthi et al., 2024). The goal of this study is to describe the fiber characteristics of the early cut whole-crop rye silages and intensively grown, annual, high sugar grass silages (*Lolium multiflorum* and *Festulolium*-type hybrids) compared to the traditional silages of dry and hot lands of Central Europe (corn silage and alfalfa silage).

Materials and methods

Samples derived from large scale farms (average farm size: 410 dairy cows/farm in 2023) in Hungary between 2013-2022 (whole-crop rye silage cut before heading, n =1379; intensive grass silage n= 910). Average milk yield was 10,683 kg milk (305 days) in 2023 in Hungary. Results are based on routine laboratory NIR analyses (Livestock Performance Testing Ltd, Hungary). Samples were dried at 70°C and ground according to the Guidelines of Samplinq® system (Eurofines Agro, Wageningen, The Netherlands, L54 2009/152). Spectra were determined according to the guidelines of NEN-EN-ISO 12099 (Q-Interline Quant FT-NIR analyser, ISO 12099:2010 guidelines for the application of near infrared spectrometry). Measured parameters are given in Table 1.

Results

Fiber digestibility (NDFd₄₈) of rye silages and high sugar grass silages (Table 1) were 66%NDF and 65%NDF, respectively compared to corn silage 61±1.6%NDF (2023, n=414) and alfalfa silage (40±7.1%NDF, 2013-2022, n= 3043). According to our results, the dNDF₄₈ content of the whole-crop winter rye silages and high sugar grass silages (cut at early stage) were higher by approximately 50-60% as compared to the corn silage (dNDF₄₈). The high NDF-digestibility is generally associated with more fast-pool, less slow-pool of NDF and less uNDF₂₄₀ -content (undegradable NDF). The undegradable ration of total aNDFom (Table 1) was rather low in early spring cut silages (uNDF₂₄₀ early-cut rye silage 20.6%NDF, intensive grass silage 23,1%NDF). Corn silage had similar uNDF₂₄₀ ratio (25.1%NDF), while the alfalfa silage had poor results (51± 6.0%NDF). We have found (Table 1) that the uNDF₂₄₀ content of the early-cut rye silages as grass silages were similar to the corn silage (uNDF₂₄₀ 112 g/kg DM - early cut whole crop rye silage; 115 g/kg DM, intensive grass silage; 100 g/kg DM corn silage 2023, n=414) and far less compared to alfalfa silage (uNDF₂₄₀ 217, 2013-2022, n= 3,043).

Table 1 Fiber fractions, fiber digestibility and undigestible fiber of early cut whole-crop rye silage (n= 1379) and early cut intensive grass silage (n=910) within the period of 2013-2022 based on 1000 g dry matter (NIR database, LPT Ltd.)

	aNDFom ¹	ADF	ADL	NDFd ₃₀ ²	NDFd ₄₈ ²	dNDF ₄₈ ³	uNDF ₂₄₀ ⁴	uNDF ₂₄₀ ⁴
	g	g	g	%NDF	%NDF	g	%NDF	g
Early cut whole-crop rye silage								
Mean	544	319	26	53	66	353	20.6	112
SD	72.0	55.3	8.1	6.5	6.5	39.3	3.5	28.5
RMSEP _{yc} ⁵	16.74	10.77	3.91	-	14.57	-	-	-
BIAS ⁶	1.26	0.11	0.38	-	-0.31	-	-	-
R ² ⁷	0.97	0.97	0.94	-	0.93	-	-	-
Intensive early cut grass silage								
Mean	499	301	25	54	65	322	23.0	115
SD	58.9	39.5	8.2	8.1	7.7	35.3	5.4	36.9
RMSEP _{yc}	15.0	8.63	3.79	-	14.01	-	17.48	-
BIAS	-2.31	-0.31	-0.13	-	-1.12	-	-1.35	-
R ²	0.94	0.93	0.80	-	0.95	-	0.71	-

¹aNDFom₄₈ - amylase treated and ash corrected NDF, ²⁻³NDFd_{30, 48} - amylase treated and ash corrected NDF rumen degradability (30 or 48 hours *in vitro* incubation- NIR data), ⁴iNDFd₂₄₀ - amylase treated and ash corrected NDF rumen undegradability or undegradable NDF (240 hours *in vitro* incubation- NIR data). ⁵RMSEP_{yc} -the actual prediction error on the validation dataset, ⁶BIAS- the average difference between predicted and actual value, ⁷R²—coefficient of determination

Conclusions

We can conclude that early-harvested rye silages and intensive grass silages have optimal digestible fiber ratio (dNDF₄₈ 65, 66%NDF, respectively) containing more digestible fiber (NDFd₄₈ 353 g kg⁻¹ DM, 323g kg⁻¹ DM, respectively) than corn silage and lucerne silage, and have similar or less indigestible fiber ratio and content (uNDF₂₄₀ 112 g kg⁻¹ DM 115 g kg⁻¹, respectively) compared to corn silage and lucerne silage. Based on these results we can project that early cut rye silage (harvested in boot stage or earlier) and intensive grass silages (*Lolium multiflorum*, Festulolium-type grass hybrids) may reduce the drop in DM-intake during the summer preventing many diseases and production losses in hot area. We suggest to introduce a new concept - the *heat stress forage* or *heat stress silage* based on fiber digestion characteristics described by parameters of dNDF₄₈, NDFd₄₈ and uNDF₂₄₀ on dry continental low land of Europe. The concept of *heat stress silage* helps to understand the positive effects on animal health and improved cow performance on farms when the proportion of corn silage in *summer TMR* is reduced from 30% to 15%, the proportion of alfalfa silage is reduced to 0-5% (compared to 10-15% overall), while the ratio of early-cut rye silage and intensive grass silage is increased from 5-10% to 15-20% overall.

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Effects of Silage Made from Fungicide-treated Grass on Dairy Cows' Performance

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Keywords: mycotoxins, silage intake, milk quality

Introduction

Higher productivity of dairy cattle places higher demands on feed quality. One hazard in feeds is mycotoxins, the secondary toxic metabolites produced by various naturally occurring fungi. They can reduce feed intake and productivity, cause reproductive problems, immunosuppression and even death. In addition, toxins can potentially be transmitted through animals to meat and dairy products. Fungal activity and mycotoxin production could be affected by type of tillage (ploughing vs. minimal tillage), and be promoted by delayed harvest, slow filling of the silo, inadequate compaction of the silage material, air leaks into the silo, and poor silage face management during feeding. Treating grasses with fungicides during the growing season could be a preventive measure to reduce the abundance of moulds on the green forage being ensiled and the content of mycotoxins in the silo. The aim of this trial was to determine the effects of silage made from grass treated with the fungicide on the productivity, milk composition, quality and safety of dairy cows.

Materials and methods

The trial was carried out with six multiparous Holstein lactating dairy cows (DIM 99±11.5; BW 674±7.9 kg). Basal values for measurable traits were determined after a 14-day preliminary period. The cows were then paired according to their lactation number, body weight, DIM, milk yield; and then randomly divided between control and treatment groups. Thereafter a cross-over design was conducted with two 3-wk periods. Data collection took place at the end of each period over five (feed intake data) and three (milk data) consecutive days. Cows were weighed at the beginning of each trial period, after silage feeding in the morning. Animal use and care was in accordance with the Estonian Animal Protection Act. The bale silage used in the trial was prepared from first cut grass harvested from the fourth year of use of the grassland. The grass used to prepare the treatment group silage was treated with the fungicide Juventus® 90 (active ingredient metconazole) with a John Deere R962i sprayer 27 days before harvest, according to the consumption rate indicated on the product sheet. Authorization was approved by the state authority for the use of fungicide. The grass used to prepare the control group silage was not treated. Feed rations (Table 1) were composed according to Estonian feeding recommendations. Cows were fed twice a day, initially with concentrate and mineral-vitamin feed for one hour and then with silage. The amounts of feed were equally divided between feeding times. Before feed change the cows were weighed and recorded. Representative feed samples were taken during the preliminary period and each trial period, they were then analysed for chemical composition. The mycotoxins deoxynivalenol (DON) and zearalenone (ZEA) were analysed by the ELISA method (R-Biopharm AG test kits). Limits of detection of DON and ZEA are 200 ppb and 50 ppb respectively.

Table 1. Ingredients and chemical composition of experimental diets in g kg⁻¹ dry matter

Ingredients	Control group	Treatment group
Silage	482	482
Concentrate [†]	500	500
Mineral-vitamin feed [‡]	18	18
Chemical composition		
Crude protein	158	160
Ether extract	41	38
Crude fibre	161	157
Metabolisable energy (MJ)	11.1	11.1
Metabolisable protein	95	95

[†] 80% barley meal and 20% heat-treated rapeseed cake.

[‡] 50% mineral-vitamin mix (Ca 225 g kg⁻¹, P 20 g kg⁻¹, Na 100 g kg⁻¹, Mg 50 g kg⁻¹, Fe 2,000 mg kg⁻¹, Zn 6,000 mg kg⁻¹, Mn 3,000 mg kg⁻¹, Cu 800 mg kg⁻¹, Se 50 mg kg⁻¹, I 150 mg kg⁻¹, Co 50 mg kg⁻¹, vitamin A 500,000 IU kg⁻¹, vitamin D3 80,000 IU kg⁻¹, vitamin E 2,000 IU kg⁻¹), 25% calcium carbonate and 25% sodium chloride.

Cows were milked twice a day with a bucket milking system. At the end of each trial period, for three consecutive days, milk yield was measured and representative milk samples (100 mL⁻¹) from each cow and from each milking were collected. Milk samples were pooled for each cow from the evening and morning milk samples in proportions according to the yields from the two milkings. Milk composition, somatic cell counts and urea contents were measured on the same day by Estonian Livestock Performance Recording Ltd with a milk analyzer CombiFoss 7DC (Foss Electric, Hillerød, Denmark). On the following day, in parallel, pH and curdling properties were measured, respectively with a pH-meter (SevenMulti; Mettler Toledo GmbH, Greifensee, Switzerland) and a lactodynamograph (Foss Italia S.r.l., Padova, Italy). For metconazole residue detection silage and milk samples were stored until the end of the trial

at -20 °C and then analysed by liquid chromatography-mass spectrometry (Agilent Technologies, Waldbronn, Germany) using LC-QToF-MS method. Statistical analysis was made by the program R (version 4.2.2) using a general linear mixed model, taking into account group, period, sampling date, animal and measured traits' basal values.

Results

Metconazole contents in the treated grass were: before harvest, after wilting and in silage respectively $183 \pm 0.2 \text{ ng g}^{-1}$, $339 \pm 9.2 \text{ ng g}^{-1}$ and $657 \pm 30.7 \text{ ng g}^{-1}$. In the control grass samples and in silage no metconazole was detected. DON and ZEA contents were below detection limits for both treated and untreated grass samples. Treatment silage contained no DON at the detection limit, while ZEA was found at 118 ppb. Control silage contained DON and ZEA at, respectively, 320 ppb and 213 ppb. The treatment cows consumed, significantly, 1.2 kg more dry matter than the control cows (Table 2) and this was related to higher silage dry matter intake (+1.0 kg). Due to the higher intake the cows produced more milk and milk solids. Their milk also had better cheese-making properties; curd firmness at 30 minutes after adding rennet was 2.3 mm thicker. The milk was safe, as the metconazole content remained below detection limit and did not exceed the allowed maximum imposed by EU Commission Regulation No. 777/2013 (0.02 mg kg^{-1} or 20 ng g^{-1} , which was equated to 20 ng mL^{-1} ; in this trial the detection limit was 5.2 ng mL^{-1}).

Table 2. Least square means of DM intake, and milk yield, composition and curdling properties

Items	Control group	Treatment group	SE	p value
Diet DM intake, kg d^{-1}	22.0	23.2	0.78	< 0.001
Silage DM intake, kg d^{-1}	10.4	11.4	0.47	< 0.001
Concentrate DM intake, kg d^{-1}	11.2	11.4	0.19	NS [†]
Mineral-vitamin feed DM intake, kg d^{-1}	0.4	0.4	0.01	NS
Milk yield, kg d^{-1}	34.1	35.3	0.46	0.017
Energy corrected milk yield, kg d^{-1}	31.6	33.3	1.21	0.033
Fat yield, kg d^{-1}	1.13	1.21	0.061	T
Protein yield, kg d^{-1}	1.17	1.22	0.036	0.008
Lactose yield, kg d^{-1}	1.64	1.69	0.036	0.018
Fat, g kg^{-1}	33.5	34.6	1.71	NS
Protein, g kg^{-1}	34.6	34.9	0.38	T
Lactose, g kg^{-1}	47.9	47.7	0.13	NS
Urea, mg L^{-1}	184	187	15.1	NS
Somatic cell count x 1,000 cells mL^{-1}	18.0	15.4	2.73	T
Metconazole, ng mL^{-1}	< LoD	< LoD	-	-
pH	6.68	6.68	0.005	NS
Coagulation time, min	11.6	11.4	0.29	NS
Curd firmness after 30 min, mm	34.3	36.6	0.86	0.025

[†] NS = not significant, T = trend ($p < 0.1$), LoD = limit of detection (5.2 ng mL^{-1})

Conclusions

The results of this trial support the use of pre-ensilage treatment of growing grass with a fungicide in practice, as it improved silage intake and milk performance, as well as the cheese-making ability of the milk. However, this requires further research to ensure legislative changes, as currently in the EU fungicides can only be used in cereal crops.

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Extraction of Metagenomic Quality DNA from Corn and Alfalfa Silage

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Keywords: silage, metagenomics, DNA extraction

Introduction

Silage is a globally important, fermented livestock feed made from forage. The chemically complex, variable silage environment can make the extraction of metagenomic-quality, representative DNA suitable for long-read, shotgun metagenomic sequencing challenging. This work details a flexible, scalable protocol for the extraction of high molecular weight, intact silage microbial DNA with negligible inhibitor carryover alongside two other methods. Of the three methods profiled, the novel method had the highest yield, longest average and maximum fragment lengths, and was the only one that was compatible with downstream Oxford Nanopore Sequencing.

Materials and methods

Wilted, chopped corn and alfalfa were collected in 2022 from the USDA-ARS U.S. Dairy Forage Research Center research dairy farm in Prairie du Sac, WI, USA. 500 grams of fresh material was placed in three replicate vacuum bag silos, inoculated with 1.0×10^7 cfu *L. plantarum*, sealed, and ensiled for 90 days. Silage bags were opened briefly to obtain samples for physicochemical analyses, resealed, and frozen at -20C prior to use for DNA extraction.

Silage samples for DNA extraction were each separated into fresh, dried-ground, and flash frozen-ground sub-samples. Sub-samples were then prepared for each of the applicable extraction protocols: unaltered commercial kit (Control), CTAB-buffer treatment prior to commercial kit (CTAB), and press treatment prior to commercial kit (Novel). Dried-ground and flash frozen-ground sub-samples were extracted with the Control and CTAB protocols; fresh silage was used for the Novel protocol.

Quality of DNA extracts was assessed by fluorometric determination of double-stranded DNA mass, spectrophotometrically, and by electrophoretic fragment analysis. Long-read DNA sequencing was performed on an Oxford Nanopore Gridion flowcell.

Results

DNA extract quality varied strongly with sub-sampling and extraction protocol. Flash frozen-ground material performed poorly for both Control and CTAB protocols. Overall, the novel method produced DNA extracts with consistently higher total double-stranded DNA mass (1.5x the next highest yield in corn; 1.8x the next highest yield in Alfalfa), average fragment length, and lowest fragment length distribution variability. In addition, DNA sequencing of extracts from the novel method had the highest number of reads and longer read lengths as compared to Control or CTAB methods. In addition, both Control and CTAB extracts resulted in an accelerated rate sequencing pore death and reduced sequencing activity.

Conclusions

The method presented here compares favorably to existing methods in terms of total DNA yield, fragment length, quality, and Oxford Nanopore long-read sequencing performance. In addition, the novel method can scale up to accommodate up to 1kg of input material, which has implications for total potential yield and reduced sampling bias. However, the novel method is more time-consuming and has lower throughput as compared to the compared protocols. We expect that this method will be of particular value for microbial shotgun metagenomics in silage and other challenging environmental samples.

References

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Influence of heat stress and ensiling temperature on growth and performance of silage inoculants

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Keywords: Silage inoculant, Climate Change, Lactic acid bacteria

Introduction

Most lactic acid bacteria (LAB) species found in silages have optimal growth temperatures near 30 °C, while many spoilage organisms have higher optimal growth temperatures and may grow at temperatures greater than 45 °C. The disparate growth conditions between desirable LAB and spoilage clostridia raise concerns regarding rising ambient temperatures and the potential effects on ensiling. The objectives of this trial were to explore the plasticity of LAB growth temperature optima and study the patterns of fermentation between silages inoculated with LAB previously exposed to high- and low- levels of heat stress and corresponding ensiling temperatures.

Materials and methods

Inoculants in liquid media incubated for 24h at 30°C (low heat stress: LHS) and 40°C (high heat stress:HHS) to test growth. Inoculants were cryopreserved in glycerol at -80°C and then revived to assess retention of any observed response to prior heat stress.

Results

All inoculants showed significant inhibition of growth in liquid culture at 40°C compared to 30°C. Inoculants were applied to mini silos of whole plant corn incubated at 30°C and 45°C. Inoculant 11 had the lowest observed pH when ensiled at 45°C regardless of prior heat stress level, but was significantly lower after HHS. Despite their poor performance in liquid culture, inoculants 6, 7, 10 and 11 all showed significant improvement in silage pH following prior high-heat stress.

Conclusions

Prior exposure to heat stress produced varied effects on the performance of silage inoculants in liquid culture and during ensiling. Exposure to HHS resulted in lower silage pH values and varied fermentation profiles for some inoculants when compared to LHS or uninoculated controls. The results of the current study provide initial evidence that heat acclimatization of silage inoculants warrants further investigation for industrial and scientific applications.

References

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Modeling Forage Conservation in the Ruminant Farm Systems (RuFaS) Model

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Keywords: Farm Systems, Model, Feed Storage

Introduction

The Ruminant Farm Systems (RuFaS) model combines knowledge of management, soils, crops, animal nutrition and husbandry, and weather to predict farm productivity, nutrient cycling and loss, energy and water use, and production costs. By predicting both production and environmental impact under diverse management and climate conditions, RuFaS provides a modular platform to assess whole system impacts of management strategies and new technologies. RuFaS uses four modular biophysical modules that simulate inputs, transformations, losses, and outputs of nutrients (N,P,K), carbon, and water in the main parts of a dairy farm: animal and housing, manure, crop and soil, and feed storage. Here we present the feed storage module's role as a nutrient-resolved, dynamic inventory of purchased and farm-grown feed and forage. Feed may be added via purchase (animal module) and from on-farm harvest (crop and soil module) and feed is removed when it is fed out in the animal module. The feed storage module currently accepts corn (silage, dry/high-moisture grain), alfalfa, grass, cereal/small grain, and soybean to be stored dry (hay, grain, and concentrates), ensiled (in bag, bunker, or pile), or as baleage. Stored feed quantity and quality changes are simulated over the duration of storage depending on crop type, crop quality, user-specified storage conditions, and ambient climate conditions. Feed component values, proportions, digestibility, and availability subject to change during storage are: mass, dry matter (DM) content, crude protein, non-protein nitrogen (NPN), neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, and water soluble carbohydrates (WSC). Forage and/or feed quality can also be user-specified both before and after storage, offering flexibility and modularity of user needs. These changes must be tracked to accurately reflect the remaining feed and its qualities. Stored feed can be used directly or be assigned a quality rating to facilitate ration formulation in the animal module.

Materials and methods

The Feed Storage Module, as well as the wider Ruminant Farm Systems model, is coded in Python and is in development for open source launch in June 2025. Mathematical functions for forage and feed quality changes during storage are a combination of empirical models and those used in the Integrated Farm Systems model, modified to accommodate a daily time-step and remove explicit spatial components.

Results

The Feed Storage Module accurately simulates the storage of farm grown feed simulated in the crop and soil module dry, ensiled, or as baleage. Simulated dry matter losses are partitioned across forage nutritional components according to the forage type, starting forage qualities, storage parameters, ambient weather and temperature, and time. Stored crops and feed accurately represent losses and changes in feed quality and are connected the RuFaS animal module to dynamically predict animal performance from feed input.

Conclusions

The Feed Storage Module of the RuFaS model acts as a bridge between field and crop simulations and animal simulations allowing for both robust whole-farm system modelling as well as interrogating feed storage-specific management consequences.

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Bacterial Diversity in Corn Silage and Total Mixed Ration Silage with or Without Microbial Inoculant

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Keywords: Lactic acid bacteria, *Lactobacillaceae*, 16S rRNA.

Introduction

Corn silages are widely recognized for their excellent quality when prepared under optimal conditions. However, exposure to air during the aerobic phase can result in significant nutrient losses. This issue arises because lactic acid, produced during fermentation, and residual water-soluble carbohydrates (WSC) in the silage serve as substrates for undesirable microorganisms. To mitigate these losses, microbial inoculants such as *Lentilactobacillus buchneri* have been extensively studied. According to the meta-analysis by Arriola et al. (2020), these inoculants enhance the population of lactic acid bacteria, increase acetic acid production contribute to improved aerobic stability and suppression of spoilage microorganisms.

The concept of total mixed ration (TMR) was introduced to optimize animal production by reducing labor costs, ensuring a balanced diet, and minimizing selective feeding by animals (Owen & Howard, 1965). Ensiling corn as part of a TMR has additional benefits, such as increasing dry matter (DM) content, diluting the WSC concentration across the diet's ingredients, and extending aerobic stability (Bueno et al., 2020). Given this context, the present study hypothesizes that ensiling corn in a TMR format enhances aerobic stability compared to conventional corn silage. The objective of this study was to evaluate the effects of ensiling corn as TMR, with or without *L. buchneri* inoculation, on the bacterial diversity.

Materials and methods

The experiment was conducted at the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (latitude 20°45'14" S, longitude 42°52'54" W). Corn was harvested 120 days after planting. Plants had a DM content of 29.85 g/kg of fresh matter. The experimental design was completely randomized, arranged in a 2 × 2 factorial scheme, with two silage types (S) and the presence or absence of inoculant (I), with five replicates per treatment. The silage types included corn silage (CS) and total mixed ration silage (TMR). The inoculants used were: control without inoculant (CONT) and a commercial inoculant containing *Lentilactobacillus buchneri* (LB) (LaSil AS, CNCM I-4323, Lallemand Animal Nutrition®, Brazil).

After harvesting, samples in the LB treatments received the inoculum at a concentration of 3×10^5 colony-forming units (CFU)/g, with a dose of 0,001 g/kg of fresh matter, while samples in the CONT treatments received distilled water (5 mL/kg of fresh matter). The TMR formulation consisted of ground corn, soybean meal, urea, mineral premix, sodium bicarbonate, and magnesium oxide, with a roughage-to-concentrate ratio of 40:60. The formulation was designed for finishing beef cattle.

After 90 days of fermentation, silage samples were collected to evaluate bacterial diversity. The DNA extraction process followed a manual method developed in-house by BymyCell® (Ribeirão Preto-SP, Brazil) using buffer solutions and magnetic beads. The MiSeq Sequencing System (Illumina Inc., San Diego, CA, USA) was used for the sequencing process. The V3-V4 region of the 16S rRNA gene was amplified using 341F (5'-CCTACGGGSGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') primers. Paired-end sequencing was performed using V3 600-cycle kits, sequencing (2x250bp, average coverage of 50,000 paired-end reads per sample). The sequences were analyzed using the Sentinel pipeline. The DADA2 pipeline was then employed to identify the taxa and generate the Amplicon Sequence Variant approach for taxonomic characterization.

Data were analyzed using a completely randomized design in a 2 × 2 factorial scheme (S × I). Statistical significance was set at $P \leq 0.05$. Fisher's F-test was used for the analysis of variance, followed by Tukey's test for pairwise comparisons. The microbial diversity data were further analyzed using the *glht-multicomp* function.

Results

In corn silages (CS+CONT and CS+LB), a greater abundance of the genera *Weissella*, *Leuconostoc*, *Klebsiella*, and *Lactococcus* were observed. In contrast, the TMR silages were characterized by greater abundances of the genera *Carnobacterium*, *Klebsiella*, *Lactococcus*, and *Lactiplantibacillus*. After 90 days of fermentation, a greater relative abundance of the genera *Lactobacillus* and *Lentilactobacillus* was detected in corn-only silages, whereas *Weissella* predominated in TMR silages.

The results of the alpha diversity analysis are presented in Figure 1. Figure 1a and Figure 1b represent the alpha diversity indices Chao1 and Shannon, respectively, for the different groups after 90 days of fermentation (CS+CONT; CONT+LB; TMR+CONT; and TMR+LB). No significant difference was observed for the Chao1 alpha diversity index ($P \geq 0.05$), which ranged from 31 to 41 (Figure 1a). However, a significant effect ($P \leq 0.05$) of the inoculant application was detected, with greater Chao1 values observed in the inoculated silages (Figure 1d). TMR silages exhibited greater ($P \leq 0.05$) bacterial community alpha diversity based on the Shannon index (Figure 1c). Nevertheless, no interaction effect ($P \geq 0.05$) between inoculant application and silage type was observed (Figure 1e). The beta

diversity analysis, based on principal coordinate analysis (PCoA), revealed significant differences ($P \leq 0.05$) among silage types, as well as an interaction effect for inoculant inclusion (Figure 1f).

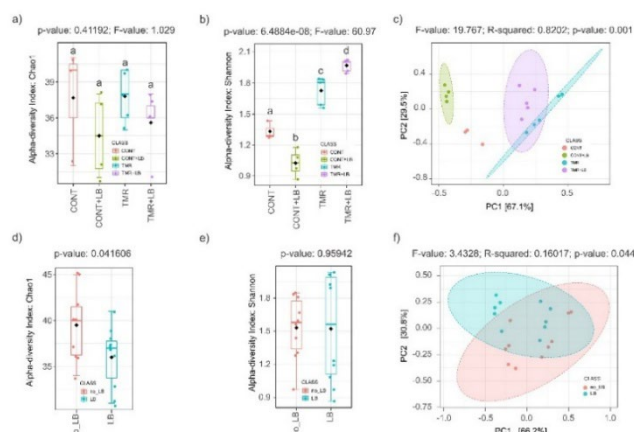


Figure 1. Alpha diversity based on the Chao1 index (a, d), Shannon index (b, e), and beta diversity (c, f). P -values for comparisons are indicated above each graph and were obtained using ANOVA with Tukey's test (a, b), F-test (d, e), and PERMANOVA (c, f). Silages were evaluated after 90 days of fermentation under control conditions (CONT), corn silage (CS), total mixed ration (TMR), and treatments with *Lentilactobacillus buchneri* CNCM I-4323 inoculant (Lallemand Animal Nutrition), referred to as CS+LB and TMR+LB.

Conclusions

The greater relative abundance of *Weissella* and *Lactiplantibacillus* in the TMR silage indicates distinct fermentation dynamics. *Weissella* is associated with early fermentation and acetic acid production, which may improve aerobic stability. In contrast, *Lactiplantibacillus* is linked to efficient lactic acid fermentation and rapid pH reduction, reflecting better preservation. The increased presence of *Lactobacillaceae* with *L. buchneri* addition further supports improved stability. These results highlight the benefits of TMR silage with microbial inoculants for enhancing fermentation quality. These findings support the use of TMR silage based on whole-plant corn with the addition of a microbial inoculant.

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Characterization of the Ruminal Microbiome of Beef Cattle Fed Total Mixed Ration Silage

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Keywords: Bacterial community, forage fermentation, metagenomics.

Introduction

It is recognized that roughage proportion, nutritional composition, and methods for diet conservation are factors that alter the ruminal microbiota (Zhang et al., 2021). Considering the ruminal environment complexity and its microorganisms' interactions, the characterization of microorganisms by conventional methods is challenging since most ruminal microorganisms are not cultivable in common media (Morgavi et al., 2013). Therefore, microbial population analysis using techniques such as metagenomics emerges as an alternative for ruminal microorganism characterization (Morgavi et al., 2013; Kibegwa et al., 2023). By identifying the microbial populations involved in fermentation processes, it is possible to evaluate their roles in the degradation of different diet components, as well as their end fermentation products. This knowledge allows us to manipulate the diet composition to favor ruminal health and, consequently, animal performance. Thus, this study aimed to characterize the ruminal microbiome of beef cattle fed with total mixed ration (TMR) ensiled or not-ensiled, with two forage proportions.

Materials and methods

Four rumen-cannulated F1 Nellore x Angus steers (initial BW of 300 ± 16 kg, and mean age of 14 ± 2 months) were assigned to a 4 x 4 Latin square design with a 2 x 2 factorial arrangement of two forage:concentrate ratios (F:C; 20:80 and 80:20, on a DM basis), and two processing types, ensiled TMR (TMRs), or not-ensiled (TMRn). The experiment lasted 80 days, with 4 periods of 20 days each, 14 days for dietary adaptation, and 6 days for data collection. Ruminal digesta was collected to characterize the bacterial populations. Genomic DNA was extracted from the liquid phase composed of a pool of ruminal samples of each animal (n=16). Raw sequence analyses were performed using RStudio (v4.3.2 R Core Team 2023) and treated to remove low-quality sequences and chimeras. The taxonomic assignment was performed using the SILVA database (v132; Quast et al., 2012). Amplicon Sequence Variants (ASVs) were clustered by 99% similarity. Bacterial community analyses were performed using the Phyloseq 1.24.2 package in RStudio. Data were normalized and graphical interfaces were obtained using the Microbiome Analyst online server (<https://www.microbiomeanalyst.ca/>). For Beta diversity, Principal Coordinate Analysis (PCoA) based on the Bray-Curtis distance was calculated and compared by the PERMANOVA test. Linear discriminant analysis effect size (LEfSe) was used to identify taxonomic groups classified at the genus level that differed significantly in abundance between the treatments.

Results

We did not observe effects ($P > 0.05$) when the processing type was analyzed (i.e., ensiled or not-ensiled). The relative abundance of bacterial taxa at the genus level showed a predominance of *Prevotella*, *Rikenellaceae_RC9_gut_group*, and, Christensenellaceae_R7_group for all treatments analyzed, where percentages averaged 18.7%, 11.43%, and 5.45%, respectively.

Evaluating the β -diversity we observed an effect ($P < 0.009$) for F:C ratio, with the axes varying from 10.5 and 13.6%, in which two distinct and well-defined groups can be verified (Figure 1a). Among the genera identified by Lefse analysis, those that presented LDA scores above 2 for the 20:80 F:C ratio were *Lachnospiraceae_NK3A20_group*, and *Atopobium* (Figure 1b). Related to 80:20 ratio, the genera identified were *Candidatus_Soleaferrea*, *Family_XIII_UCG_001*, *Pseudobutyrvibrio*, *Lactobacillus*, *Shuttleworthia*, *Fibrobacter*, *Butyrivibrio*, *Acetobacter*, *Lachnospiraceae_ACC2044_group*, *Lachnospiraceae_XPB1014_group*, *Candidatus_Saccharimonas*, *Saccharofermentans*, and *Succiniclasticum* (Figure 1b).

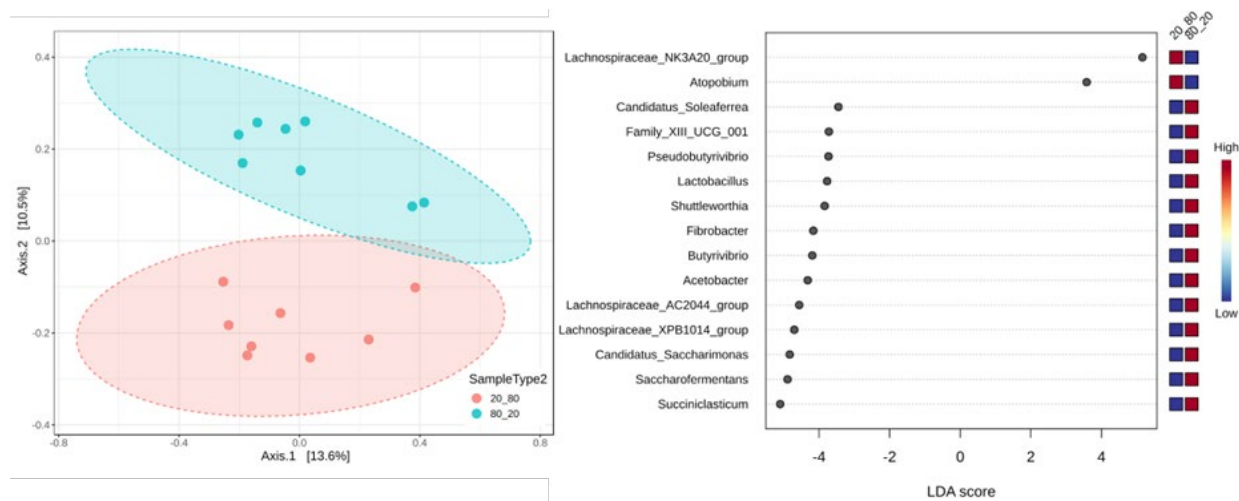


Figure 1. Beta diversity analyses were performed using principal component analysis (PCoA), comparing the different forage:concentrate ratios (a). Lefse analysis shows the taxa representing the significant differences for the different forage:concentrate ratios for the liquid phase, with the F:C ratio 20:80 in red and 80:20 in blue (LDA Score >2; (b).

Conclusions

The relative abundance of bacterial taxa at the genus level in the liquid phase showed a predominance of *Prevotella*, *Rikenellaceae_RC9_gut_group*, and *Christensenellaceae_R7_group* for all treatments analyzed. The predominance of bacterial populations in treatments with greater forage-to-concentrate ratios highlights the dietary influence on ruminal microbiota. Presenting valuable insights for developing dietary strategies for the optimization of ruminal bacterial activity and achieving animal performance improvements.

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Intake and Digestibility of Beef Cattle Fed Corn Silage or Total Mixed Ration

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Keywords: Lactic acid bacteria, nutritive value, starch.

Introduction

Optimizing livestock production systems has been a central focus of recent research, aiming to enhance precision, efficiency, and sustainability. Adapting diets to meet specific nutritional requirements of animals has been effective in reducing waste, labor, and costs. In this context, total mixed ration (TMR) silages have emerged as a promising strategy in ruminant production due to their operational and nutritional advantages, such as uniform ingredient mixing, reduced feed selection by animals, strategic grain conservation, and the inclusion of by-products (Owen & Howard, 1965).

TMR silage also demonstrate greater aerobic stability, maintaining feed quality over time (Nishino et al., 2003). In corn silage, extended fermentation periods enhance starch digestibility due to proteolysis of the protein matrix surrounding starch granules (Nair et al., 2020). Similar improvements are anticipated in TMR silage, where the interaction between moisture and concentrates enhances protein solubility and nutrient availability (Hao et al., 2020).

The use of microbial inoculants, such as *Lentilactobacillus buchneri*, improves silage quality by reducing dry matter losses, minimizing ethanol production, and promoting the activity of lactic acid bacteria during the initial stages of fermentation (Nair et al., 2020).

It was hypothesized that TMR silages influence intake and digestibility in beef cattle. Thus, the objective of this study was to evaluate the effects of TMR corn silages, with or without *L. buchneri* inoculation, on the intake and digestibility of nutrients in beef cattle.

Materials and methods

The experiment was conducted at the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil. The corn crop was harvested 120 days after sowing, with a dry matter (DM) content of 29.85 g/kg of fresh matter. A 4 × 4 Latin square design was used, with four animals, four diets, and four experimental periods. The treatments followed a 2 × 2 factorial arrangement, including two silage types [corn silage (CS) and total mixed ration silage (TMR)] with or without (CTRL or LB) microbial inoculant.

The inoculant used was a commercial product containing *Lentilactobacillus buchneri* (LB) (LaSil AS, CNCM I-4323, Lallemand Animal Nutrition®, Brazil). After harvesting, the material was treated with LB at a concentration of 3 × 10⁵ colony-forming units (CFU)/g or with CTRL, consisting of distilled water (5 mL/kg). The TMR formulation included ground corn, soybean meal, urea, mineral premix, sodium bicarbonate, and magnesium oxide, with a roughage-to-concentrate ratio of 40:60, formulated for finishing beef cattle. Both CS and TMR were ensiled in concrete silo tubes and stored for 90 days.

The experimental period lasted 80 days, divided into four 20-day periods, with 14 days for diet adaptation and six days for sample collection. Feed intake and digestibility were determined by the difference between feed offered and refusals, complemented by fecal sampling. Data were analyzed using analysis of variance (ANOVA) based on the 4 × 4 Latin square design arranged in a 2 × 2 factorial scheme. Fisher's F-test was applied, considering a significance level of $P \leq 0.05$.

Results

No significant effects ($P \geq 0.05$) of silage type (S), inoculant (I), or their interaction (S × I) were observed on the intake of dry matter, organic matter, or starch, with average values of 7.34 ± 0.93, 6.93 ± 0.87, and 2.87 ± 0.43 kg/day, respectively. However, an effect of silage type ($P = 0.0338$) was observed on neutral detergent fiber intake, with lower value recorded for TMR silage (1.94 kg/day) compared to CS (2.26 kg/day).

An interaction effect (S × I) was detected ($P = 0.0464$) for organic matter digestibility (Table 1). Animals fed TMR silage exhibited higher digestibility of dry matter ($P = 0.0011$), crude protein ($P = 0.0130$), and starch ($P = 0.0028$) compared to those fed CS (Table 1). Neutral detergent fiber digestibility was not influenced ($P \geq 0.05$) by any of the factors studied, with an average value of 56.51 ± 1.25%.

Table 1. Nutrient digestibility of diets fed to finishing beef cattle based on whole-plant corn silage (CS) or total mixed ration silage (TMR), with (*L. buchneri*; LB) or without (CTRL) microbial inoculant.

Inoculant	Silage		P-value				
	SM	TMR	Average	SEM	S	I	S × I
Dry matter (%)							
CTRL	71.06	77.03	74.04	1.689	0.1538	0.0011	0.0545
LB	64.08	78.34	71.21				
Average	67.57	77.68					
Organic matter (%)							
CTRL	72.60Ba	78.33Ab	75.46	1.634	0.1239	0.0010	0.0464
LB	65.48Bb	79.51Aa	72.49				
Average	69.04	78.92					
Crude protein (%)							
CTRL	63.01	68.70	65.85	2.448	0.1877	0.0130	0.1156
LB	51.54	69.91	60.72				
Average	57.27	69.30					
Starch (%)							
CTRL	84.81	90.93	87.87	1.005	0.7992	0.0028	0.8780
LB	84.25	90.79	87.52				
Average	84.53	90.84					

SEM: Standard error of the mean; P-value: Probability of effects for inoculant (I), silage type (S), and their interaction (I × S); Means followed by different uppercase letters in rows and lowercase letters in columns differ significantly according to Fisher's F-test ($P \leq 0.05$).

Conclusions

Corn silage, fed alone or as part of a total mixed ration, does not significantly affect the nutrient intake of beef cattle. However, its inclusion as a component of TMR silage increases the digestibility of dry matter and starch, providing a practical advantage in the daily feeding of animals using ensiled TMR.

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A New Aeration Method to Test Aerobic Stability in Silage Trials

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Keywords: Homofermentative bacteria, heterofermentative bacteria, *L. buchneri*, *L. plantarum*, silage additive, aerobic stability.

Introduction

Testing silage additives for aerobic stability is sometimes challenging due to slow aerobic deterioration of silages in laboratory settings. Predetermined aeration (exposure to air) of laboratory silos is performed in order to challenge silages and speed up spoilage processes. Traditionally, jars with defined openings are used to apply aeration challenge. We tested a new method of aeration, not requiring special jars.

The aim of this study was to study the effects of a new aeration method on fresh matter weight loss and aerobic stability.

Materials and methods

A silage trial using 1.5 L glass jars (600g FM per jar) was conducted over 49 days including 5 groups with 3 repetitions each for aerated and continuously closed groups (2 x 5 factorial design). Groups were treated as follows: 1 = control without additives; 2 = 94% *Lentilactobacillus buchneri* (*L. buchneri*), 6% *Lactiplantibacillus plantarum* (*L. plantarum*); 3 = 85% *L. buchneri*, 15% *L. plantarum*; 4 = 70% *L. buchneri*, 30% *L. plantarum*; 5 = 100% *L. plantarum*. Extensive grass was ensiled with a sugar content of 2.7% in FM and a dry matter (DM) content of 40.6 %. All silage additives (groups 2-5) were applied at a concentration of 300.000 CFU/ g fresh matter (FM). On day 28 and 42, aerated jars were aerated with the new method: clamps were opened, the rubber was loosened and the jar was turned upside down for 24h. Thereafter, the jars were turned upright, closed again and tightly sealed. Continuously closed jars were not aerated or turned and left close until day 49. Before and after aeration as well as before and after opening on day 49, jars were weighed with a precision scale. Aerobic stability was tested according to DLG TestService GmbH (2020). Test was interrupted after 29d after opening and samples that did not show a temperature rise until this day were determined to have aerobic stability of 29d.

Results

Inoculation treatment affected weight loss in continuously closed and aerated groups until day 49 with highest losses in groups 1 (0.74% FM and 0.76% FM, respectively) and 2 (0.62% FM and 0.73% FM,

Table 1. Weight loss of grass silage on day 49 after ensiling including 5 groups (1 = control group without additives, and groups 2 to 5 with varying proportions of *L. buchneri* / *L. plantarum*: 2 = 94/6, 3 = 85/15, 4 = 70/30, 5 = 0/100). Aerated samples were opened on days 28 and 42 for 24h, continuously closed samples were not opened until day 49.

		Group = 1		Group = 2		Group = 3		Group = 4		Group = 5	
Aeration		Closed	Aerated	Closed	Aerated	Closed	Aerated	Closed	Aerated	Closed	Aerated
Weight loss until 49d	g	4.45 ^a (1.57)	4.54 ^a (0.29)	3.72 ^{a,b} (0.14)	4.40 ^{a,b} (0.20)	3.34 ^{a,b,c} (0.09)	3.96 ^{a,b} (0.09)	2.94 ^{b,c} (0.03)	3.67 ^{a,b} (0.08)	2.16 ^c (0.07)	3.51 ^{a,b,c} (0.06)
Proportional weight loss until 49d	% FM	0.74 ^a (0.26)	0.76 ^a (0.05)	0.62 ^{a,b} (0.02)	0.73 ^{a,b} (0.03)	0.56 ^{a,b,c} (0.01)	0.66 ^{a,b} (0.02)	0.49 ^{b,c} (0.01)	0.61 ^{a,b} (0.01)	0.36 ^c (0.01)	0.59 ^{a,b,c} (0.01)
Weight loss during opening 49d	g	0.12 ^a (0.06)	0.03 ^{a,b,c} (0.01)	0.07 ^{a,b,c} (0.08)	-0.05 ^c (0.03)	0.06 ^{a,b,c} (0.05)	-0.02 ^{b,c} (0.03)	0.013 ^{a,b,c} (0.006)	-0.017 ^{b,c} (0.023)	0.09 ^{a,b} (0.04)	0.08 ^{a,b,c} (0.06)
Proportional weight loss during opening 49d	% FM	0.020 ^a (0.010)	0.004 ^{a,b,c} (0.001)	0.012 ^{a,b,c} (0.014)	-0.008 ^c (0.005)	0.009 ^{a,b,c} (0.008)	-0.003 ^{b,c} (0.005)	0.0022 ^{a,b,c} (0.0010)	-0.0028 ^{b,c} (0.0038)	0.014 ^{a,b} (0.006)	0.014 ^{a,b,c} (0.009)
Aerobic stability 49d	d	23 ^{a,b} (10)	2 ^{c,d} (1)	29 ^a (0)	22 ^{a,b,c} (12)	29 ^a (0)	4 ^{b,c,d} (2)	17 ^{a,b,c,d} (10)	3 ^{c,d} (0)	29 ^a (0)	1 ^d (0)

a, b, c, d Treatments with the same letter are not significantly different ($p > 0.05$)

respectively) and lowest losses in group 5 (100% homofermentative additive, 0.36% FM and 0.59% FM, respectively, Table 1). Aerated samples lost 0.02% (Control group) to 0.23% FM (Group 5) more weight than continuously sealed samples. Aerobic stability was high in continuously sealed groups (17d to 29d). Aerobic stability decreased in all aerated jars and was significantly lower in aerated jars in groups 1, 3, and 5 compared to continuously closed jars of these groups (Table 1).

During aeration on day 28, samples lost between 0.86 g (Group 5) and 1.17 g (Group 2) or between 0.143% and 0.195 % FM (Figure 1) within 24h aeration period. During aeration on day 42, weight loss was lower than on day 28 and significantly lower in groups 2 to 4 (0.17 to 0.20 g, $p < 0.05$) than in the control group (0.39 g). In groups 2 to 4 (94 to 70% *L. buchneri* in silage additive), a vacuum was present during opening on day 49 in aerated groups, which was confirmed by weight increase (negative weight loss) after loosening the rubber and opening the lid (Table 1 and Figure 1).

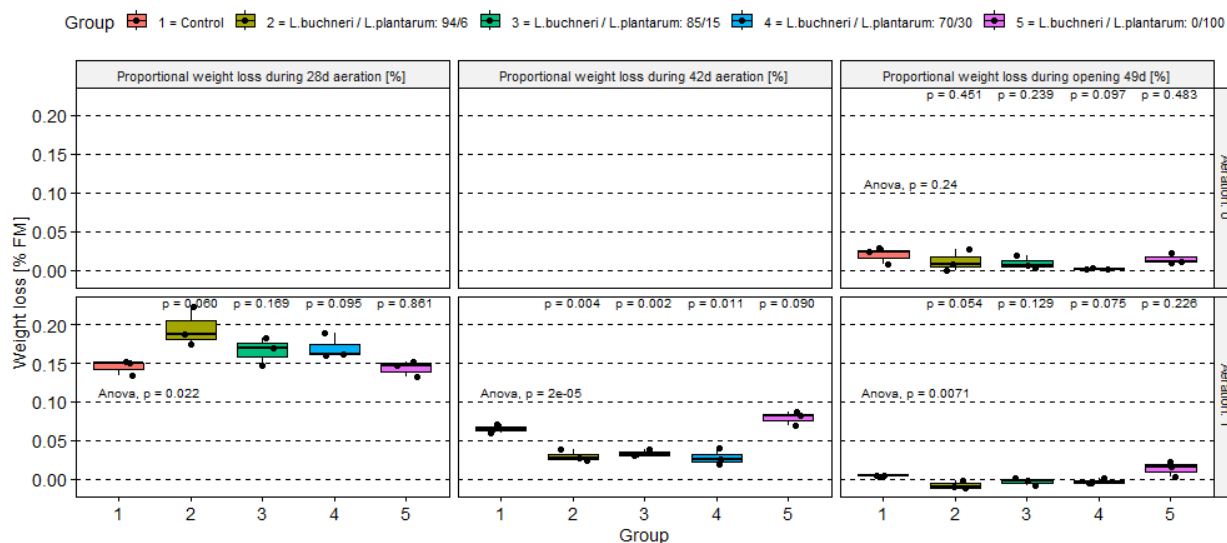


Figure 6 Effect of silage additives on proportional weight loss [% of fresh matter (FM) of initial weight on day 0] during aeration with new method for 24h on days 28 and 42 and during opening on day 49 after ensiling (upper row = continuously sealed samples; bottom row = aerated samples). Aeration was realized by removing the clamps from the jar, loosening the lid and turning the jars upside down for 24h. After aeration, jars were tightly sealed again. Groups 2 to 5 vary in proportions of *L. buchneri* and *L. plantarum*, e.g. group 2 was inoculated with 94% *L. buchneri* and 6% *L. plantarum* at 300,000 CFU/g fresh matter. Control group without additives.

Conclusions

The new aeration method was effective in causing aerobic challenge to laboratory silages. Weight loss during opening times on days 28 and 42 was observed, indicating that CO₂ might have been exchanged by ambient air. Weight change during aeration was dependent on silage additive. A vacuum was observed during opening on day 49 in groups containing *L. buchneri*, which might indicate that gas was assimilated by microorganisms in these jars after aerobic challenge. Among all groups, continuously sealed jars had the lowest weight loss compared to aerated jars. Groups containing 100% *L. plantarum* had the lowest weight loss within aeration groups.

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Use of Optical pH-Measurement in Early Silage Fermentation Trials

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Keywords: pH-value, homofermentative bacteria, heterofermentative bacteria, *L. buchneri*, *L. plantarum*.

Introduction

Testing pH-value within the first few days of silage fermentation trials gives valuable information about the ensiling process. However, samples cannot be taken without opening the experimental jars, requiring additional repetitions per desired sampling time. Optical, non-invasive pH-measurement has been developed by PreSens Precision Sensing GmbH (Regensburg, Germany) and tested in various experimental settings (Koop-Jakobsen et al., 2018, Reinders et al., 2019). The objective of this pilot study was to assess, whether optical pH measurement with self-adhesive sensor spots (PreSens Precision Sensing GmbH, Regensburg, Germany) is feasible for the use in laboratory silage trials.

Materials and methods

Sensor spots were attached to the inner bottom of 0.5 L glass jars. The trial was conducted over 4 days including 6 treatment groups with 3 repetitions each ($n = 18$). Silage treatments were: 1 = untreated control, 2 = 94% *Lentilactobacillus buchneri* (*L. buchneri*) / 6% *Lactiplantibacillus plantarum* (*L. plantarum*), 3 = 85% *L. buchneri* / 15% *L. plantarum*, 4 = 70% *L. buchneri* / 30% *L. plantarum*, 5 = 50% *L. buchneri* / 50% *L. plantarum*, 6 = 0% *L. buchneri* / 100% *L. plantarum*. Inoculants were applied at 3×10^5 CFU / g fresh matter (FM). The different treatments were used to obtain a more diverse fermentation dynamics and final pH range. Grass (second cut, mostly perennial ryegrass (*Lolium perenne*) and oat-grass (*Arrhenatherum elatius*), was ensiled with a sugar content of 5.5% in FM and a dry matter (DM) content of 39.0%. On day 0 and day 4, pH was measured in aqueous solution as described in Tabacco et al. (2009) and on day 4 with an insertion electrode (FiveEasy Plus, Mettler-Toledo GmbH, Gießen, Germany). Additionally, on days 0 to 4, pH was measured daily by optical measurement using pH-1 SMA LG1 (PreSens - Precision Sensing GmbH, Regensburg, Germany) through the glass wall of the jar. The three pH-measurements on day 4 were compared using Pearson correlation coefficient.

Results

Between 6h and 24h after ensiling, a steep decrease in pH-values was observed using the optical pH measurement (Figure 1). Thereafter, pH-values decreased only slowly until day 4. However, standard deviations of optical measurement were greater than in aqueous solution. On day 4, correlation between optical pH-measurement and measurement in aqueous solution was moderate but significant at $r = 0.53$ ($p = 0.025$). Correlation between pH-values measured with insertion electrode and aqueous solution was only weak at $r = 0.31$ ($p = 0.22$) and between insertion electrode and optical pH measurement there was a weak correlation at $r = 0.16$ ($p = 0.54$).

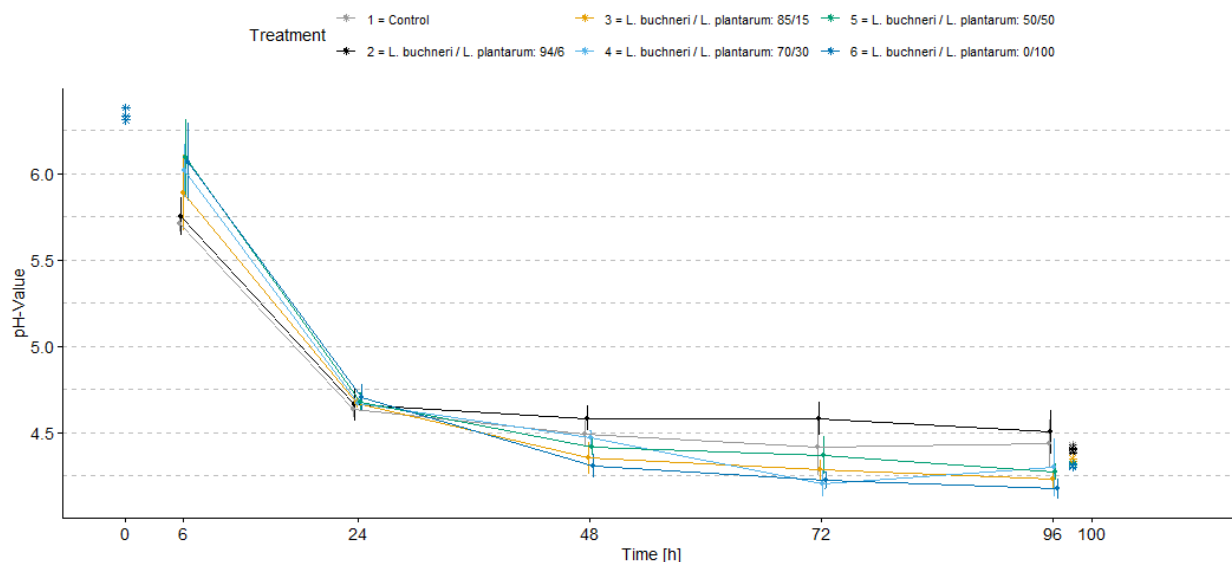


Figure 7 pH-value measured in a silage trial over 4 days. Measurements at 0h and 96h (stars) were taken in aqueous solution and measurements at 6h, 24h, 48h, 72h, and 96h (lines) were taken using an optical probe. Treatments 2-6 varied in the proportions of *L. buchneri* and *L. plantarum* in silage additive e.g. treatment 2 was comprised of 94% *L. buchneri* and 6% *L. plantarum*.

Conclusions

Optical pH measurement is a novel opportunity to describe the ensiling process, especially the early phase of fermentation. The advantage is that for pH-measurement, the laboratory silo does not have to be opened and more data points can be measured with fewer replicates. The description of the pH in the early fermentation phase could be performed, for example, every minute, if desired. On the other hand, the ensiling material is inhomogeneous and

therefore, the surface touching the sensor spot might have a range of pH-values. Further studies should test this new optical pH-measurement technique using different dry matter contents, chopping lengths and ensiling materials.

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Chemical Composition and *In vitro* Rumen Degradability of Crude Protein in Whole Crop Hemp Silages

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Keywords: *Cannabis sativa* L., rumen degradability, silage additive

Introduction

The use of whole hemp plants applies to industrial, nutritional, and medicinal purposes. Because hemp plants contains a lot of crude fiber, which is only partially degraded during ruminal fermentation, its value in ruminant nutrition may be limited. The assessment of ruminal degradability is a key element in evaluating the suitability of fibrous feeds for ruminants. The aim of the study was to evaluate the degradation of crude protein in silage made from *Cannabis sativa* L., produced with or without the addition of preservatives.

Materials and methods

The silages were produced from fresh material with low THC content, obtained from a plantation in northeastern Poland. The hemp, at the stage of seed formation, was harvested with a precision cutter at a height of 20 cm and ensiled in airtight barrels: Hemp – hemp silage without additives; Hemp+SA – hemp silage with a commercial chemical additive with acidifying properties; Hemp+M – hemp silage with molasses at a dose of 2-4%. After a 4-month fermentation period, the silage samples were collected and the content of basic nutrients was determined: dry matter (DM), crude ash (CA), crude protein (CP), crude fat (CF), crude fiber (CFb) (AOAC, 2016); neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Van Soest et al., 1991); water-soluble carbohydrates (WSC) (Thomas, 1977); lactic acid, acetic acid, and butyric acid by gas chromatograph (GC). The pH of the silages and the content of ammonia nitrogen (N-NH₃) were determined using the direct distillation method. The ruminal degradability of silage CP was determined *in vitro* by the gravimetric method according to Trujillo et al. (2010). The silage samples were incubated in ruminal fluid, collected from 5 fistulated Kamieniecka sheep, for 0h, 2h, 4h, 8h, 24h, and 48h. The CP degradation was calculated according to Kowalski et al. (2008). The results were analyzed using ANOVA ($p < 0.05$), followed by Duncan's post-hoc test to identify significant differences between treatment groups.

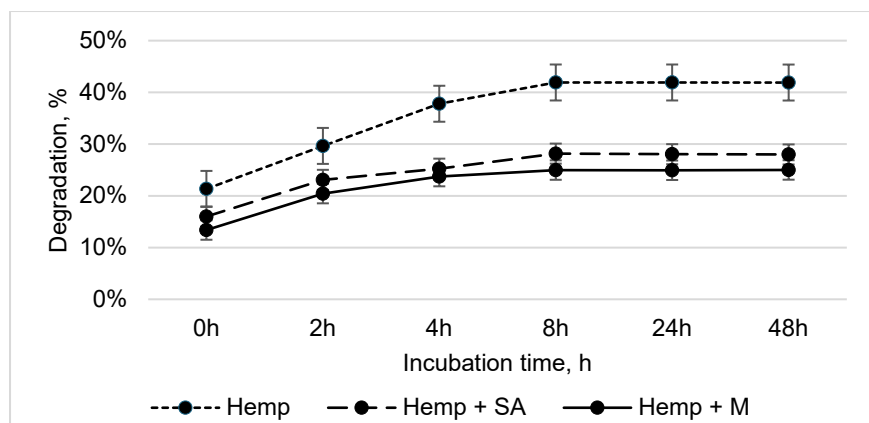
Results

The silages differed significantly in terms of CA, CP, CF, CFb, as well as the NDF, ADF, and ADL fractions ($P \leq 0.05$). Hemp silage, ensiled without additives (Hemp), had significantly lower contents of CA, CP, and CF compared to the other silages ($P \leq 0.05$). On the other hand, the Hemp+SA and Hemp+M silages showed significantly lower concentrations of CFb, NDF, and ADF, with the highest concentration of ADL ($P \leq 0.01$). The addition of SA or M to the hemp silage did not affect the fermentation profile. All silages were characterized by limited lactic fermentation with high N-NH₃ concentration. The low content of butyric acid ($<1\%$) indicates the high quality of the hemp silages (Table 1). The hemp silages differed in ruminal degradability *in vitro* for CP from 4 hours to 48 hours of incubation (Figure 1). The lowest final ruminal degradability of CP was observed in the Hemp+SA silage (28%) and the Hemp+M silage (25%). The effect of the SA and M additives in hemp ensiling on the reduction of *in vitro* CP degradability at 8h, 24h, and 48h incubation of the silages was confirmed. The initial incubation time, from 0h to 2h, was similar for all silage variants.

Table 1. Chemical composition and fermentation pattern (g kg⁻¹ DM) of silages

Item	Silage			SEM	P- value
	Hemp	Hemp + SA	Hemp + M		
Dry matter, g kg ⁻¹ fresh matter	406,40	413,90	423,60	0,325	NS
Crude ash	49,80 ^b	59,80 ^{ab}	67,10 ^a	0,276	**
Crude protein	61,10 ^b	74,10 ^a	77,50 ^a	0,291	*
Crude fat	12,70 ^b	35,50 ^a	37,10 ^a	0,400	***
Crude fiber	561,20 ^a	492,90 ^b	466,20 ^b	1,506	**
NDF	727,90 ^a	677,50 ^b	651,60 ^b	1,221	**
ADF	649,40 ^a	580,90 ^b	549,90 ^b	1,574	**
ADL	70,60 ^b	80,60 ^{ab}	85,60 ^a	0,954	**
WSC	10,60	8,50	8,70	0,056	NS
pH	5,20	5,29	5,20	0,039	NS
N-NH ₃ , g·kg ⁻¹ N _{total}	137,07	148,99	140,59	0,376	NS
Lactic acid	54,43	50,83	50,06	4,348	NS
Acetic acid	4,16	3,23	3,23	0,309	NS
Butyric acid	0,46	0,82	0,40	0,889	NS

SEM – standard error of the mean; NS -not significant ($P > 0.05$); values marked with the same letters (a - b) are not significantly different at the $P \leq 0.05$ level; * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001



Incubation time, h	0h	2h	4h	8h	24h	48h
P-value	ns	ns	*	**	**	**

Figure 1. Degradation of crude protein during *in vitro* incubation

Conclusions

The use of SA and M additives in the ensiling process of hemp limited the *in vitro* degradation of total protein in the rumen. The observed differences in chemical composition, as well as in ruminal protein degradability, could, apart from the effect of preservatives, also result from the lack of uniform structure of the plants. Preparing homogeneous silage samples was problematic because of the morphological structure of the plants. The effect of the preservatives may have also differed depending on the specific parts of the plant (leaves, stems).

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The Effect of Ensiling Lucerne with Birdsfoot Trefoil at Different Ratios on Protein Fractions

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Keywords: birdsfoot trefoil, protein fractions, lucerne silage

Introduction

Lucerne (*Medicago sativa* L.) is one of the basic plant materials for the production of silage in Europe. In lucerne silage, various types of additives are often used, because it has a specific difficulty in fermentation. Birdsfoot trefoil (*Lotus corniculatus* L.) has a high tannin content among legumes, which may be a factor in improving the protein and fermentation quality of lucerne. The aim of the study was to analyse the quality of fermentation and protein of silage made from lucerne and birdsfoot trefoil at different ratios.

Materials and methods

Plant materials were harvested at the beginning of bud formation stage, a height of 5 cm, with a disc mower (12:00-1:00 p.m.). It was cut to a particle (25 mm) and allowed to wilt for 12 hours. Lucerne was mixed with birdsfoot trefoil in the proportions: 100:0 (control group), 90:10, 70:30, 50:50. The experimental variants were ensiled in micro-silo (1 dm³) in triplicates for 90 days. Dry matter (DM), crude protein (CP), extract ether (EE) (AOAC, 2016), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) (Van Soest et al., 1991) and water soluble carbohydrate (WSC) (Thomas, 1977) were determined. Silage samples were also analyzed to determine pH, the content of lactic acid (LA) by high performance liquid chromatography (HPLC), ammonia nitrogen (N-NH₃) by direct distillation and short-chain fatty acids (SCFA): acetic acid (AA), propionic acid (PA), isobutyric acid (iBA), butyric acid (BA), isovaleric acid (iVA), valeric acid (VA) by gas chromatograph (GC). The protein fractions were analyzed according to Licitra et al. (1996). The CP distribution was done according to The Cornell Net Carbohydrate and Protein System (CNCPS) v 6.5 model described by Higgs et al. (2015) and Van Amburgh et al. (2015). The results were statistically processed by performing an analysis of variance (ANOVA) at a significance level of 0.05, Duncan's or Dunn's test and principal component analysis (PCA) using the XLSTAT package.

Results

Co-ensiling of both plants affected changes in carbohydrate composition (Table 1). An increase in the proportion of birdsfoot trefoil in lower NDF (P≤0.05) and WSC (P≤0.01) content in the 50:50 silage in compared to control group. There was an increase in N-NH₃ concentration in the 50:50 silage compared to the control silage. The silage of the 50:50 had the highest concentration of AA, PA and SCFA (P≤0.001). In the results presented in Figure 1, as the proportion of birdsfoot trefoil in the silages increased, there was a significant increase in the PA1 fraction (P≤0.001), and a decrease in the PA2 fraction (P≤0.001). An increase in the valuable PB1 (P≤0.05) and PB2 (P≤0.01) fractions indicates an improvement in the CP quality of the prepared silages with a higher proportion of birdsfoot trefoil (30% and 50%). PCA analysis showed that the first group of variables correlated with each other and at the same time with high values for the silages of the control group (100:0) were NDF, WSC and the PB1 fraction. The second group including 50:50 silages were DM, EE, N-NH₃, AA, PA, SCFA, PA1 and PC fractions. For the final third group comprising 90:10 and 70:30 silages, no correlations were found with the parameters analyzed.

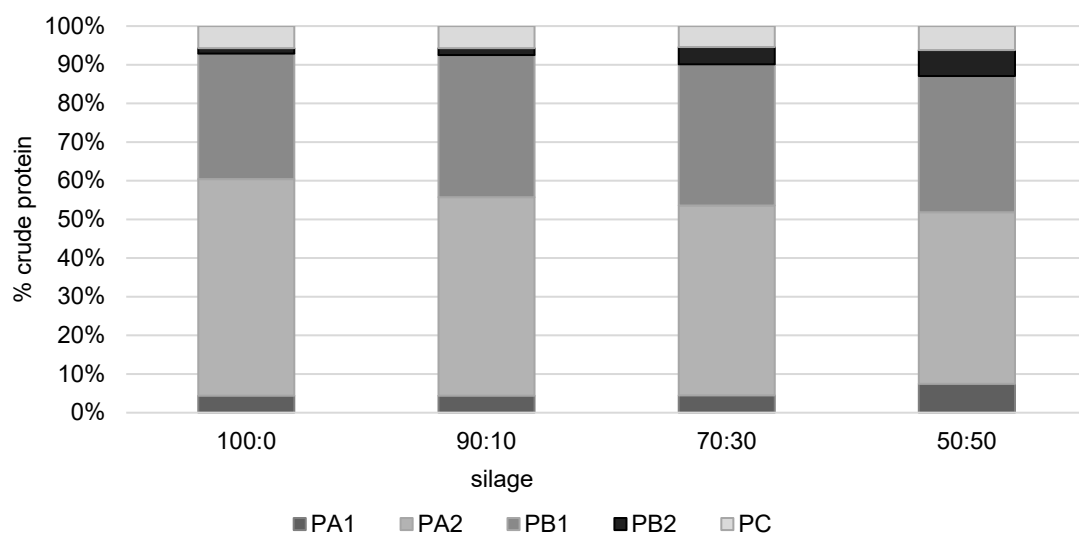
Conclusions

The experiment showed that co-ensiling alfalfa with birdsfoot trefoil caused changes in fermentation products and protein fractions, in favor of an increase in the valuable PB1 and PB2 fractions, which was confirmed statistically. The course of lactic fermentation was not altered, and the obtained values of chemical parameters should be considered correct with respect to literature data. Both the 30% and 50% proportion of birdsfoot trefoil reduced proteolysis during ensiling of alfalfa, which could be due to the activity of the tannins contained in it. The ensiling of alfalfa with a 50:50 proportion of birdsfoot trefoil can be the basis for the production of very good quality silage with high protein quality.

Table 1. The chemical parameters of experimental silage (g·kg⁻¹ DM)

Item	Silage				P	R ²
	100:0	90:10	70:30	50:50		
Dry matter	321	321	324	328	n/a	0.544
Crude protein	196	190	195	198	n/a	0.221
Extract ether	19.8	18.5	20.2	21.9	n/a	0.436
NDF	465 ^a	422 ^{ab}	401 ^b	393 ^b	*	0.657
ADF	367	350	339	338	n/a	0.573
ADL	60.8	60.7	61.4	64.4	n/a	0.555
WSC	17.2 ^a	15.0 ^a	14.1 ^a	10.5 ^b	**	0.749
pH	4.81	4.80	4.83	4.82	n/a	0.724
N-NH ₃ , g·kg ⁻¹ N _{total}	44.1 ^b	43.9 ^b	44.7 ^b	74.5 ^a	***	0.898
Lactic acid	53.8	46.3	26.7	43.4	n/a	0.498
Acetic acid	2.80 ^b	3.29 ^b	2.57 ^b	5.81 ^a	***	0.866
Propionic acid	0.14 ^b	0.12 ^b	0.12 ^b	0.28 ^a	***	0.865
Butyric acid	0.01	0.01	0.01	0.01	n/a	0.205
SCFA	2.98 ^b	3.45 ^b	2.73 ^b	6.19 ^a	***	0.875

R² - coefficient of determination; n/a - not applicable ($P > 0.05$); values marked with the same letters (a - b) are not significantly different at the $P \leq 0.05$ level; * ≤ 0.05 ; ** ≤ 0.01 ; *** ≤ 0.001



Fraction	PA1	PA2	PB1	PB2	PC
P	***	***	*	**	n/a
R ²	0.898	0.328	0.794	0.702	0.092

Figure 1. Protein fractions of experimental silage (g·kg⁻¹ CP)

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Field Monitoring of Inoculant Temperature in Tractor-mounted Applicator Tanks in Northern Mexico

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Keywords: Data loggers, Real-time measurements, Bacteria viability

Introduction

Inoculating forage crops for silage making is common in many regions of Mexico, but the methods of application vary greatly. While direct application through the forage harvester machinery is strongly recommended, this is often not the norm. Instead, inoculants are commonly applied using makeshift spraying equipment mounted on tractors responsible for packing or distributing forage in the silo. Since most tractors do not have a dedicated structure or space for these tanks, they are typically mounted on the front counterweights. However, this setup poses a significant risk to the viability of bacteria in silage inoculants because the heat radiated from the tractor engine can raise and sustain the tank's temperature above the critical threshold of 35°C (Mulrooney and Kung, 2008). This risk is further exacerbated by high ambient temperatures during the first harvest season in this arid region of Mexico. Based on these conditions, we hypothesized that silage inoculants in this type of tanks quickly gain temperature above the viability threshold and that many farm operators are unaware of this issue. Consequently, the objectives of this field study were to evaluate producer awareness of the effect of temperature on the viability of inoculant bacteria and to monitor the temperature progression in tractor-mounted applicator tanks.

Materials and methods

Three large farms participated in the field survey to monitor the temperature progression inside the inoculant tanks mounted on their tractors. Temperature monitoring was performed in four tanks under normal working conditions during the 2024 summer harvest season in the Laguna region in Northern Mexico using submersible data loggers (ThermoWorks, American Fork, Utah, USA) set to record temperature every 5 minutes. The data loggers were deposited in the inoculant tanks when they were filled for the first time in the day and were left recording between 3 and 4 consecutive days. Tractors operators were only notified of the placement of a “thermometer” and instructed to continue working normally according to their usual activities pertaining preparing and refilling the tanks. When the data loggers were retrieved the time of the day was manually recorded; the devices were taken to a safe location away from the silo, recording was stopped, and the data were downloaded using the manufacturers software (ThermaData® Studio v.6.5.0.0 ThermoWorks, American Fork, Utah, USA). The individual data from each tank was truncated to the closest actual time of immersion and retrieval. The data were inspected to identify the following parameters: initial temperature in the tank, time of day when the temperature of the inoculant reached 35°C, maximum temperature inside the tank, duration of temperature $\geq 35^{\circ}\text{C}$, time of the day when the temperature returned to $< 35^{\circ}\text{C}$.

Results

The results indicated that the minimum temperature was recorded at the beginning of the day when the inoculated tank filled for the first time and ranged between 21 and 24°C. After the initial filling of the tank, the temperature rose gradually until reaching 35°C between 12:00 and 14:00 h. The maximum temperature in most tanks was $\geq 40^{\circ}\text{C}$ between 4 and 5 hours after being filled for the first time in the day and remained above 35°C for 4-5 hours. It was also noted that refilling the tank with freshly prepared inoculant during the day lowered temperature between 3 and 4°C.

Conclusions

Tractor mounted inoculant tanks reached the critical temperature within 5 hours after filling them for the first time in day and remained above this threshold for the next 5 hours reaching internal temperature that can compromise the viability of bacteria in the inoculant. Refilling inoculant tanks reduced internal temperature slightly but not enough to return below 35°C. Therefore, it is recommended to use insulated tanks and use fresh or cold water to prepare the inoculant in this semiarid region to maintain temperature below the critical threshold to promote greater viability of bacteria in the inoculant.

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Effect of Reducing Wilting Time and Adding Corn Grains and/or Soybean Hulls on Silage Quality

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Keywords: alfalfa silage, wilting, corn grain, fermentation profile, aerobic stability

Introduction

For successful forage preservation as silage, the harvested material must be wilted. This process increases the forage dry matter (DM) content to around 35%, depending on the storage type. Wilting exposes the forage to changing weather, leads to physical losses, and induces proteolysis, ultimately reducing forage quality (Fijałkowska et al., 2015, Rooke and Hatfield, 2015, Ávila and Carvalho, 2020). A silage method that could reduce wilting time would thus be advantageous in preserving nutritional value in forage. The objective of this study is to assess the effects of reducing wilting time in alfalfa by adding corn grains and/or soybean hulls on the chemical composition, fermentation profile and aerobic stability of alfalfa silage.

Materials and methods

The study was conducted at the Agronomic Station of Laval University, Quebec, Canada using alfalfa (second cut at early flowering stage) in randomized complete block design (5 blocks, 5 plots [experimental units] per block). Five silage types were prepared, including three wilting levels: no wilted (NW, 0 h, 20% DM), partially wilted (PW, 6 h, 28% DM) and fully wilted (FW, 24 h, 35% DM). Concentrates (ground corn (GC) with or without soybean hulls (SH)) were added to a target DM content of $33 \pm 2\%$. Silage treatments were: FW, control or fully wilted (24 h) alfalfa; NW-GC, non-wilted alfalfa (0 h) with ground corn; NW-GCSH, non-wilted alfalfa (0 h) with ground corn and soybean hulls; PW-GC, partially wilted alfalfa (6 h) with ground corn; and PW-GCSH, partially wilted alfalfa (6 h) with ground corn and soybean hulls. The forage-to-corn-to-soybean hull ratios (on DM basis) were 48:52:0, 48:34:18, 75:25:0 and 75:16:9 for NW-GC, NW-GCSH, PW-GC and PW-GCSH, respectively. Treated forage was sampled, and then ensiled in experimental silos and opened after 2, 4, 8, 16, 48, and 96 days of ensiling. Silage DM, neutral detergent fiber (NDF), crude protein (CP), starch, protein fractions and fermentation profile (NH₃, acetic, lactic, propionic, and butyric acids) were determined. Aerobic stability (time to reach 2°C above the ambient temperature [h]) was measured over 10 days on silages opened at 96 days. Data were analyzed using SAS Studio with mixed-effects models. Interaction effects were first tested excluding the control treatment; since no significant interaction was detected, a priori contrasts were subsequently performed. Significant differences were declared at $P \leq 0.05$.

Results

The concentrations of crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) varied according to the concentrates added to achieve the desired dry matter content (Table 1). From day 4 (Figure 1, Table 1), silage pH was consistently lower in non-wilted silages (NW), intermediate in partially wilted silages (PW), and highest in the fully wilted silage (FW). Ammonia concentration was lower in NW than in FW and PW (104.4, 114.2 and 146.9 g/kg N total for NW, PW and FW ; $P < 0.01$, contrasts I and II). The rapidly degradable protein fraction (A+B1) was reduced in PW compared to NW (65.2 and 63.6% of CP for NW and PW, $P < 0.01$, contrasts III) and tended to be lower than FW (66.7% of CP, $P = 0.06$, contrast II). True protein fraction (B2) tended ($P=0.06$) to be higher in PW silages compared to the other treatments. Aerobic stability was lower in NW (148.9 h) compared to FW (240 h) and PW (240 h) ($P < 0.01$, contrasts I and II), and higher in GCSH (213.9 h) than GC (175 h) ($P < 0.01$, contrasts IV).

Table 10: Effect of wilting level (non-wilted NW; partially-wilted PW, or fully-wilted FW) and grain types (GC, grain-corn or GCSH, grain corn and soybean hull) on pH, chemical composition, protein fractions and aerobic stability of alfalfa silages after 96 days of ensiling.

Wilting level	Treatments					SEM	P Value	Contrasts ³			
	NW		PW		FW			I	II	III	IV
Grain type	GC	GCSH	GC	GCSH	-						
DM at ensiling, %	31.1	32.1	34.7	34.8	33.5	0.9	0.01	0.04	0.18	<0.01	0.45
pH	4.01	4.05	4.24	4.23	4.41	0.03	<0.01	<0.01	<0.01	<0.01	0.60
NH ₃ , g/kg N	105.5	103.2	116.7	111.7	146.9	8.5	<0.01	<0.01	<0.01	0.21	0.64
Chemical composition	g/kg DM										
aNDF	289.4	371.4	383.4	421.5	449.2	7.0	<0.01	<0.01	<0.01	<0.01	<0.01
Starch	247.1	155.9	88.6	67.9	8.0	9.0	<0.01	<0.01	<0.01	<0.01	<0.01
CP	137.8	140.8	158.8	161.4	176.7	5.9	<0.01	<0.01	0.03	<0.01	0.59
Protein fractions	% of CP										
AB1	66.2	64.1	63.6	63.5	66.7	1.0	0.02	0.12	<0.01	0.06	0.17
B2	24.4	24.4	27.1	26.3	24.7	0.8	0.06	0.75	0.02	0.01	0.57
Aerobic stability ⁴ (h)	110	188	240	240	240	14.4	<0.01	<0.01	0.97	<0.01	<0.01

³Contrasts: I : FW vs. NW ; II : FW vs. PW ; III : NW vs. PW ; IV : GC vs. GCSH ; ⁴Time to reach 2°C above the ambient temperature after silo opening (h)

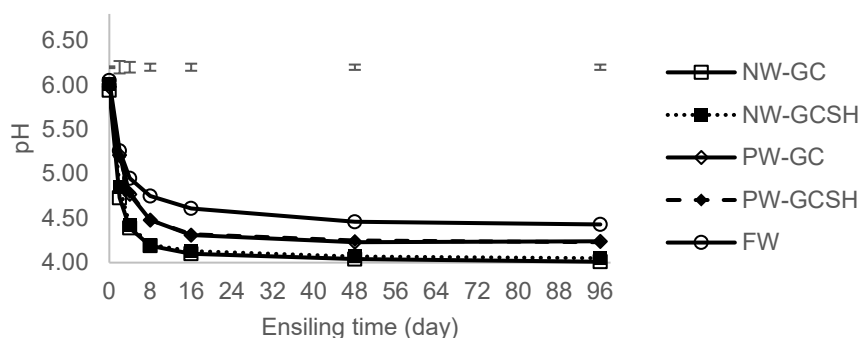


Figure 8: Effect of wilting time and concentrates addition on silage pH

Conclusions

This study highlighted the potential advantages of combining reduction of wilting time and dry concentrates additions on alfalfa silage quality. High quality silage was produced with reduced wilting time. Partially wilted silages added with grains preserved more true protein and contained less ammonia, enhancing protein quality compared to non-wilted added with grains and fully wilted silages. Aerobic stability after silo opening was compromised in treatments receiving highest amount of concentrates (non wilted silages). Overall, reducing or eliminating wilting, combined with the addition of corn grains and/or soybean hulls, offers a promising strategy for improving the efficiency of alfalfa silage production. This approach reduces the risks associated with weather conditions, while improving fermentation quality and nutrient preservation of silage. However, further research is needed to validate and optimize this method under on-farm conditions to ensure its practical applicability.

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Impact of Ensiling and Storage Temperature on the Aerobic Stability of Maize Silage

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Keywords: 9-sample-live-measuring-system, pH, temperature, aerobic deterioration

Introduction

The fermentation period and the aerobic storage period of silages result in the emission of greenhouse gases. In order to reduce the emissions and conservation losses, a detailed investigation of this process is beneficial. Previous research has described how these emissions respond to variations in the storage temperature of the feed. In stylo silage, differences of 10 K have been shown to affect the fermentation quality parameters (Liu et al., 2012). In alfalfa silage it was reported to alter the microbial population and fermentation profile (Zhang et al., 2018). As the temperature in the storage phase often fluctuates between 21°C to 30°C (Kung, 2011), it is vital to analyze the effects of different storage temperatures in detail. Therefore, the aim of this study is to investigate the effect of storage temperature during fermentation on the aerobic stability of maize silage, using a novel 9-sample-live-measuring-system.

Materials and methods

Whole plant maize was grown at the Frankenforst Campus of the University of Bonn (Königswinter, Germany; 50°42'50.1"N 7°12'24.9"E) and harvested on the 12th September 2024. The forage material (35% dry matter) was ensiled directly (CON) or inoculated (BIO) with a commercial biological additive consisting of *Lentilactobacillus diolivorans*, *Lentilactobacillus buchneri* and *Lacticaseibacillus rhamnosus*,

(Schaumann Pinneberg, Germany) before ensiling in cylindrical glass jars (1.5 L, Weck glass and packaging GmbH, Bonn, Germany). The gasses were stored either at room temperature (23°C) or in an incubator (Binder, Tuttlingen, Germany) set at 33°C. This resulted in four treatments: non-inoculated maize stored at 23°C (CON23) or 33°C (CON33) and maize treated with an additive stored at 23°C (BIO23) or 33°C (BIO33). After an ensiling period of 60 days, four jars of both temperature treatments of not inoculated silage were placed in the 9-sample-live-measuring-system (Sun et al. (2025), this volume) to continuously measure pH, temperature, oxygen (O₂) content within the silage material, as well as the gaseous emissions of carbon dioxide (CO₂) and ethanol for 2 weeks at 23°C ambient temperature. At the end of the measuring period, four jars of both temperature treatments of the inoculated silage were subjected to the same process.

Results

An established marker for aerobic deterioration of silage is the material reaching a temperature of 2°C above ambient (Wilkinson & Davies, 2013). The CON silage reached this value before the BIO treatment silage (see figure 1A). In the CON treatment, CON23 was on average $2.08 \pm 1.24^\circ\text{C}$ warmer than the ambient temperature after 58 h, whereas CON33 reached an average $2.0 \pm 1.02^\circ\text{C}$ after 65 h of aerobic storage.

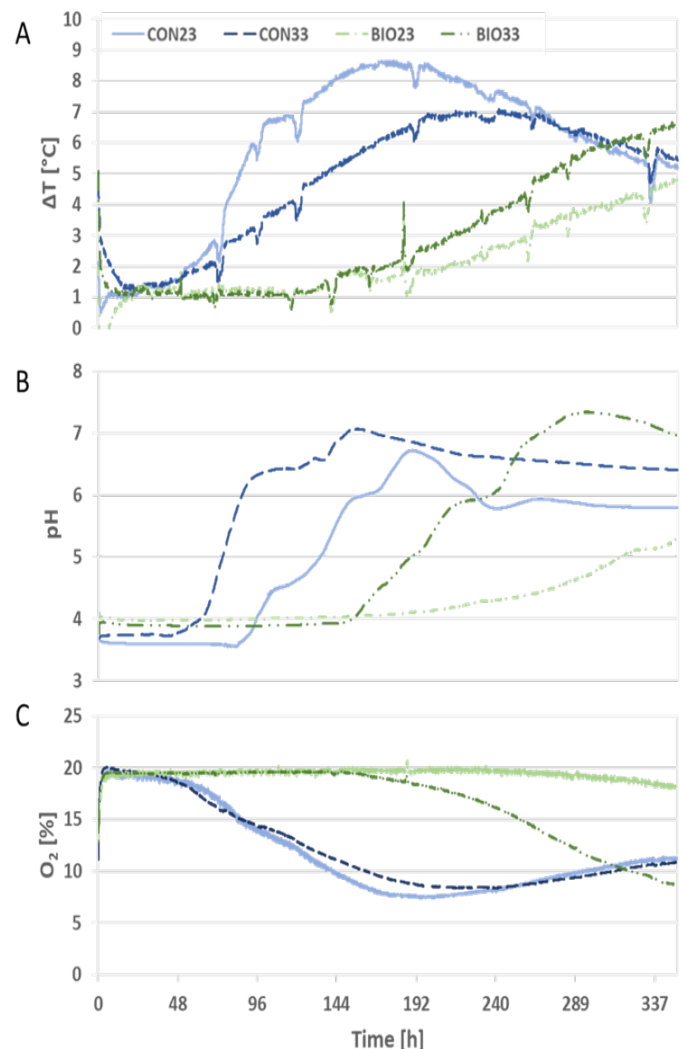


Figure 1: Real-time measurements of maize silage in aerobic storage period. (A) shows the temperature difference of the silage in comparison to ambient temperature [°C], (B) the pH-values and (C) the O₂ concentrations in the silage. The silage was either untreated or inoculated with the biological additive and anaerobically stored at two different ambient temperatures during fermentation (n = 4).

For the BIO treatment, BIO23 reaches the threshold of $2.00 \pm 1.24^{\circ}\text{C}$ at 212 h of aerobic storage, BIO33 is $2.01 \pm 1.07^{\circ}\text{C}$ above the ambient temperature at 175 h of aerobic storage. There were no significant differences in the time required to reach the threshold between the groups, most likely due to high standard deviations (see figure 2A).

The pH increased earlier in silages stored at 33°C , regardless of the treatment (see figure 1B). On average, the four repetitions of CON23 reached a pH of 6 ± 1.4 after 162 h of aerobic storage. When stored at 33°C during ensiling, the material reached a pH of 6 ± 1.4 after 87 h of aerobic storage. BIO23 resulted in an average pH not rising above 6 after 350 h of aerobic storage, whereas BIO33 required 240 h of aerobic storage to reach a pH of 6 ± 1.9 . Furthermore, it was found that the influx of O_2 into the deeper layers of the silage was suppressed earlier in silages without inoculation (see figure 1C). In both CON treatments the percentage of O_2 fell below 15% after 87 h of aerobic storage (on average

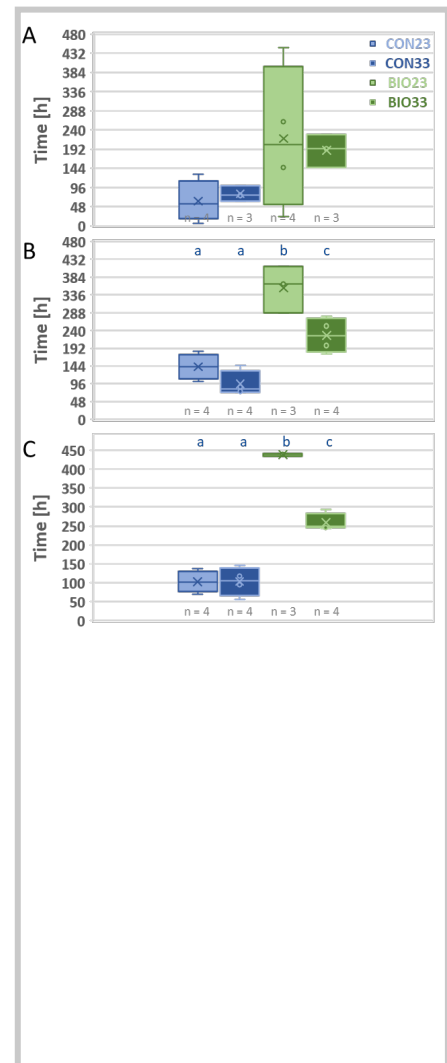
14.7 ± 3.6 for CON23, 14.9 ± 4.1 for CON33). In the BIO treatment, BIO33 required 256 h of aerobic storage for an average of $14.9 \pm 1.3\%$ O_2 . BIO23 does not go below the value of 15% O_2 after 350 h of ensiling. Significant differences were found for the time needed to reach the thresholds of pH and O_2 . No significant differences were found between the two CON groups. However, there were significant differences between the CON and BIO treatments, as well as between the two BIO temperature variants (see figure 2B and C).

Conclusions

The impact of ambient temperature during fermentation not only affects fermentation parameters as shown in previous studies, but also spoilage parameters during the aerobic storage phase of the silage. Oxygen ingress into deeper layers of the silage is an indicator for the activity of spoilage microbes in the material, as is the rise of the pH when organic acids are metabolized. Considering this, the material ensiled with the additive indicates a later increase in microbial activity when ensiled at 23°C than when ensiled at 33°C . Previous studies reveal an effect of temperature on the fermentation profiles of lactic acid bacteria strains and a higher ratio of lactic acid to acetic acid when ensiled at 30°C (Zhang et al., 2018). This would be consistent with our results showing a higher aerobic stability when ensiled at 23°C with biological additive. The measured pH indicates a clear difference in the rate of spoilage progress. On the other hand, the differences with respect to the ambient temperature do not demonstrate significant differences between temperature treatments. However, they support earlier spoilage in silage stored at 33°C , if the limit of 2°C above ambient temperature is applied.

Overall, as long as the temperature range is viable for the microbes, it can be concluded that a biological additive has a more profound effect on aerobic stability than the fermentation temperature.

Figure 2: Time until the silage (A) shows a $\Delta T > 2^{\circ}\text{C}$, (B) reached a measured pH-value of 6 and (C) the O_2 concentrations 15 cm behind the surface drops below 15%. The silage was either untreated or inoculated with the biological additive and anaerobically stored at two different ambient temperatures during fermentation (n values indicated below the boxes) Values within the same figure that have different lowercase superscripts differ significantly from each other at $P < 0.05$.



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Effect of Microbial Inoculation, Storage Temperature and Storage Length on Fermentation Profile of Whole-plant Corn Silage

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Keywords: aerobic stability, fermentation profile, nutrient composition.

Introduction

Exposure to high temperatures during fermentation can influence corn silage fermentation profile and microbial activity (Bai et al., 2022). However, research on the use of heterofermentative microbial inoculants in corn silage at high temperatures is limited and further studies are warranted. The hypothesis of this study was that greater storage temperatures would impair its fermentation, reduce aerobic stability, and increase spoilage after aerobic exposure. Additionally, microbial inoculation with a combination of *L. buchneri*, *L. diolivorans* and *P. acidilactici* would increase acetic and propionic acid concentrations, as well as aerobic stability, with these effects becoming more pronounced over longer storage periods.

Materials and methods

Whole-plant corn silage (WPCS) was harvested at UW-Arlington Agriculture Research Station at approximately 40% DM from four random locations (block) within a field. Subsamples of 15 kg from each location were assigned to 1 of the 16 treatments which were a combination of a microbial inoculant (MI) [LPLD (300,000 CFU/g whole-plant corn forage of *Pedococcus acidilactici* DSM 16243, *Lentilactobacillus buchneri* DSM 12856, and *Lentilactobacillus diolivorans* DSM 32074; Provita Supplements Inc.), or distilled water (control)], two storage temperatures (ST; 22 or 40°C) and four storage lengths (SL; 7, 15, 30 and 90 d), in quadruplicate for a total of 64 mini silos. Each laboratory silo was treated individually with spray bottles and hand-mixed immediately prior to ensiling in 20L plastic buckets, before being randomly assigned to be stored at either normal temperature (NT; 22 ± 1.52°C) or a high temperature (HT; 40 ± 2.12°C), until reaching their assigned storage length. After opening, representative samples were collected and frozen for later analysis, and the remaining material was mixed and assessed for aerobic stability, with temperature recorded every 30 min for 240h by two temperature sensors for each bucket. The environmental temperature during the aerobic stability assay matched the ST (22 or 40°C) depending on the ST treatment. Silage samples were analyzed at a commercial laboratory for fermentation profile and nutrient composition. The storage temperature, microbial inoculation, storage length, and all their interactions were considered fixed effects, and location (replicate) was treated as a random effect. Data were analyzed using the GLIMMIX procedure of SAS and means were compared by Bonferroni's test ($\alpha = 0.05$). If a three-way interaction was detected ($P \leq 0.05$), effects were partitioned by storage length using the SLICE option.

Results

Propionic acid was analyzed but not detected in any treatment. An interaction between MI × ST as well as an interaction between ST × SL were observed for pH ($P = 0.02$ and $P = 0.001$, respectively). For the interaction between MI × ST, the pH was greatest for LBLD-40 (3.94), intermediate for LBLD-22 (3.89), and lowest for CON-22 and CON-40 (3.84, on average). For the interaction between ST × SL, pH was greater for HT than NT at 30 d (3.97 vs. 3.85) and 90 d of storage (3.79 vs. 3.65). An interaction between ST × SL was observed for lactic acid concentration (Figure 1A; $P = 0.001$), which was greater for NT than HT at 30 d (4.74 vs. 3.79% DM) and 90 d (5.52 vs. 4.25% DM). A three-way interaction was observed for acetic acid concentration (Figure 1B; $P = 0.001$); at 90 d of storage, LBLD-22 was greater (4.39% DM), than CON-22, CON-40 and LBLD-40 (2.34% DM, on average). Ethanol concentration was 0.3 percentage-units lower ($P = 0.001$) for HT compared to NT. A two-way interaction of MI × ST was detected ($P = 0.001$) for 1,2-propanediol concentration, which was greatest for LBLD-40 (0.102% DM), intermediate for LBLD-22 (0.043% DM), and lowest for CON-40 (0.009% DM), while CON-22 (0.032% DM) did not differ from either LBLD-22 and CON-40. The concentration of 1,2-propanediol was 0.046 percentage-units lower ($P = 0.005$) for 7 d compared to 90 d. A three-way interaction was detected for 1-propanol concentration (Figure 1C; $P = 0.001$), which was greater for LBLD-22 than other treatments at 90 d (0.536 vs. 0.004% DM, on average). Similarly, a three-way interaction was detected for aerobic stability (Figure 1D; $P = 0.001$), which at 90 d was greatest for LBLD-22 (240 h) than LBLD-40, CON-22, and CON-40 (71 h, on average). The concentration of SCP was greater ($P = 0.001$) for HT than NT (50.9 vs. 58.2% CP). A two-way interaction of ST × SL was observed for ammonia-N concentration, which was greater for HT than NT at 7 d (3.55 vs. 2.42% N), 15 d (4.61 vs. 3.06% N), 30 d (5.93 vs. 3.54% N) and at 90 d of storage (7.93 vs. 5.36% N), but the magnitude of this effect was greater as storage length progressed. Greater StarchD was observed ($P = 0.001$) for HT than NT (71.8 vs. 69.3% starch). Starch digestibility also increased ($P = 0.001$) with SL from 67.9% at 7 d to 73.9% starch at 90 d of storage.

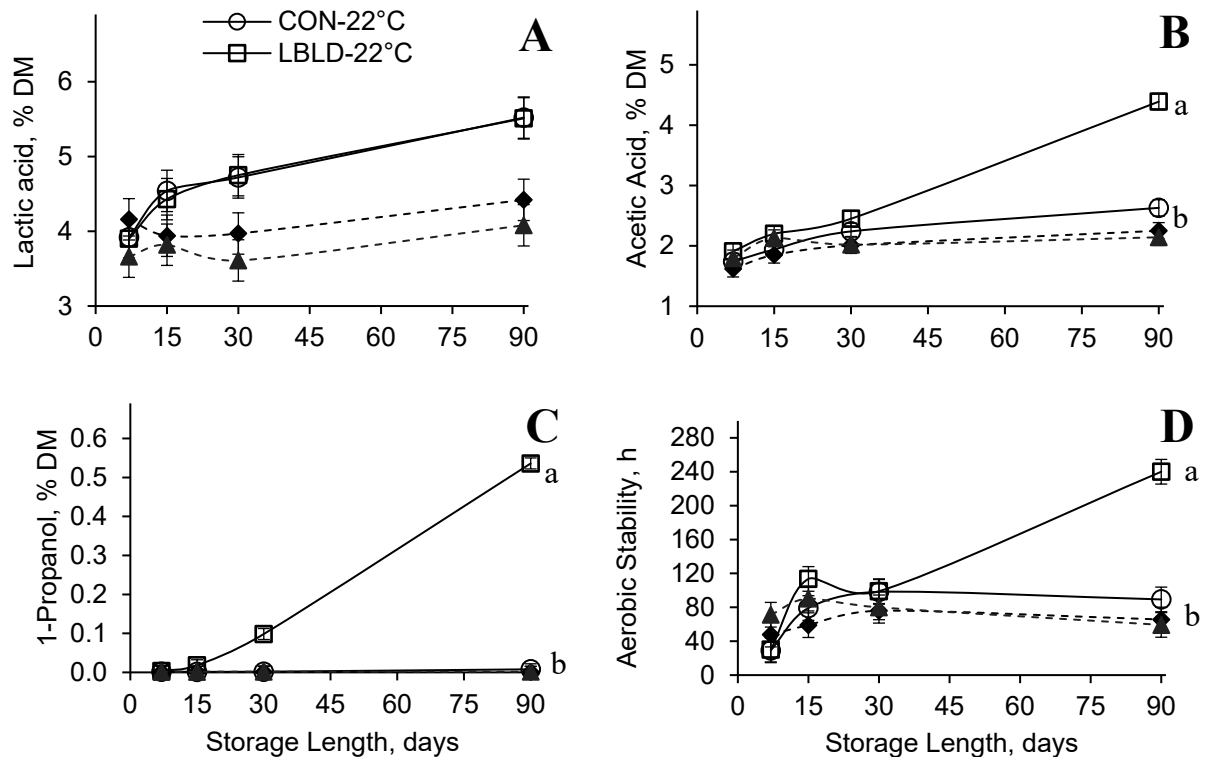


Figure 1. The effect of microbial inoculation (MI), storage temperature (ST), storage length (SL) on concentrations of lactic acid, acetic acid, 1-propanol and aerobic stability of whole-plant corn silage ($n = 64$). Means within the same day with different superscripts differ ($P < 0.05$). Whole-plant corn silage was treated with distilled water (CON) or LBLD (*P. acidilactici* DSM 16243, *L. buchneri* DSM 12856, and *L. diolivorans* DSM 32074; 300,000 CFU/g forage) and stored at different ST (22 or 40°C). Silage was stored for 7, 15, 30 or 90 d. Each treatment combination of MI, ST and SL has 4 observations. Error bars represent standard error of the mean (SEM). (A) Lactic acid, % DM; $P = 0.92$; SEM = 0.28; (B) Acetic acid, % DM; $P = 0.001$; SEM = 0.13 (C) 1-propanol, % DM; $P = 0.001$; SEM = 0.01; (D) Aerobic stability, h; $P = 0.001$; SEM = 14.55.

Conclusions

Inoculating WPCS with a heterofermentative inoculant enhanced acetic acid concentration and improved aerobic stability for silage stored under lower temperatures for 90, but not 15 nor 30 days, underscoring the effect of greater temperatures in fermentation and on the effectiveness of microbial inoculants.

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The Effects of Storage Temperature on the Nutrient Composition and Fermentation Profile of Silage Through a Meta-Analysis

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Keywords: environmental temperature, fermentation profile, nutrient composition.

Introduction

Although environmental temperature cannot be controlled during harvesting and ensiling, understanding the extent of damage that temperature may cause to the silage can be important. Such knowledge can provide insights into management strategies to reduce nutritive losses under challenging conditions (Ashbell et al., 2002). Under normal conditions, the temperature gradually decreases, depending on environmental conditions and silo size. However, in warm climates, high silage temperatures may persist for several months (Weinberg et al., 2001). In contrast, in cooler environments, the lower average daily temperature can also negatively impact the fermentation process, delaying the pH decline during the initial days after silo sealing (Zhou et al., 2016). Therefore, the objective of this study was to evaluate the magnitude of the effects of environmental temperature on nutritive value and fermentation profile of silages through a meta-analysis. The hypothesis of this study was that both low and high temperature ranges would negatively affect nutritive value and fermentation profile of silage.

Materials and methods

Literature published in the English language from 1984 to 2024 was searched using “silage temperature”, “silage fermentation temperature”, and “silage storage temperature” as keywords. The eligibility criteria included (1) evaluation of temperature within fixed or small range temperatures; (2) temperature remained constant during the storage period; (3) tested simultaneously at least two fermentation temperatures as treatment means; (4) evaluated at least 12h of ensiling period to ensure changes on silage fermentation. After initial screening, 105 peer-reviewed articles were assessed to determine their eligibility to be included in the meta-analysis, and based on the inclusion criteria, 42 peer-reviewed papers (up to 253 comparisons) were selected to analyze storage temperature (ST) effect on silage nutritive value. The number of replicates, treatment means, and sources of errors were collected from tables of the published manuscripts, and data were transferred to Excel spreadsheets (Microsoft Corp., Redmond, WA) and reviewed by two trained people. Treatment means were classified as silage type [whole-plant corn, grass, cereal, legume, total mixed ration silage, and others (crop residues, by-products, specific plant materials)]; storage length; temperature range (in groups of 10°C, reaching the following ranges: 1) $\leq 10^{\circ}\text{C}$; 2) > 10 and $\leq 20^{\circ}\text{C}$; 3) > 20 and $\leq 30^{\circ}\text{C}$; 4) > 30 and $\leq 40^{\circ}\text{C}$; 5) $> 40^{\circ}\text{C}$. Data were analyzed using a mixed model procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC). Storage length and silage type were used as continuous and categorical covariates, respectively. The model included storage temperature range as fixed effect. A random intercept was specified for each study to account for between-study variability. The covariance structure for the random effects was set to unstructured (“type=un”). Study was considered as a random effect. The weight of each study was calculated as the inverse of the square of the variance as proposed by Roman-Garcia et al. (2016). Degrees of freedom were calculated using the Kenward-Roger option. Means were determined using the LSMEANS statement and compared using the Tukey’s test option after a significant overall treatment *F* test. Orthogonal contrasts were used to evaluate linear and quadratic effects of storage temperature. Statistical significance was considered at $P \leq 0.05$.

Results

Fermentation profile, microbial counts and nutritive value of silage stored at different temperature ranges are presented in Table 1. Overall, dry matter concentration linearly increased ($P = 0.003$), whereas pH decreased linearly ($P = 0.001$) with greater ST. Lactic acid concentration quadratically increased ($P = 0.004$) as the ST range increased. Acetic acid concentration was affected ($P = 0.03$) by ST, but no differences were observed after Tukey’s test corrections. In addition, the concentration of $\text{NH}_3\text{-N}$ was greater ($P = 0.001$) for the ranges of > 20 to $\leq 30^{\circ}\text{C}$ (7.26 % N) and > 30 to $\leq 40^{\circ}\text{C}$ (7.32 % N) than > 10 to $\leq 20^{\circ}\text{C}$ (6.12 % N), while the ranges of $\leq 10^{\circ}\text{C}$ (6.53 % N) and $> 40^{\circ}\text{C}$ (6.17 % N) did not differ from them. A linear reduction was observed for LAB ($P = 0.001$), yeast ($P = 0.001$), and mold counts ($P = 0.02$), whereas ST quadratically increased WSC concentration ($P = 0.001$). Storage temperature linearly increased SCP ($P = 0.001$).

Table 1. The effects of storage temperature on the chemical (DM basis) composition of silage¹

Item ²	Temperature range, °C					SEM ³	<i>P</i> -value	Contrast ⁴	
	≤ 10	11 - 20	21 - 30	31 - 40	> 40			L	Q
Dry matter, % as-fed	30.5b	30.1b	31.0b	30.9ab	32.5a	0.732	0.001	0.003	0.34
pH	5.21a	4.52b	4.11c	4.19c	4.22bc	0.088	0.001	0.001	0.37

Lactic Acid, % DM	3.20b	3.88c	5.00a	4.04b	4.83ab	0.286	0.001	0.02	0.004
Acetic Acid, % DM	1.40	1.16	1.09	0.983	1.28	0.099	0.03	0.08	0.06
NH ₃ -N, % N	6.53ab	6.12b	7.26a	7.32a	6.17ab	0.773	0.001	0.09	0.76
LAB, log CFU/g	7.71a	7.68a	6.85b	5.54c	5.81c	0.983	0.001	0.001	0.21
Yeast, log CFU/g	4.09a	4.24a	2.87b	2.78b	0.75c	1.46	0.001	0.001	0.001
Mold, log CFU/g	2.41a	2.16a	1.47b	1.72ab	1.44ab	0.497	0.02	0.02	0.28
WSC, % DM	4.85b	2.19c	2.54c	2.45c	7.76a	0.409	0.001	0.01	0.001
SCP, % CP	-	43.3c	48.9b	53.4a	-	2.18	0.001	0.001	0.44

¹Silage was stored at different environmental temperatures and were grouped and analyzed within five ranges of temperature [1) ≤ 10°C; 2) > 10 and ≤ 20°C; 3) > 20 and ≤ 30°C; 4) > 30 and ≤ 40°C; 5) > 40°C].

²DM: dry matter; NH₃-N: ammonia concentration; LAB: lactic acid bacteria; WSC: water-soluble carbohydrates; SCP: soluble crude protein

³SEM: standard error of the mean.

⁴L: linear; Q: quadratic.

Conclusions

The environmental temperature during fermentation influences LAB activity, with both high and low temperatures impairing their normal function due to reduced total acids production. Therefore, the temperature range of 21-30°C may be more suitable for silage storage by promoting greater fermentation profile and rapid decrease in silage pH, thereby enhancing nutrient preservation.

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Effects of Whole-plant Corn Yeast Population on the Nutrient Composition, Fermentation Profile and Aerobic Stability of Silage Through a Meta-analysis

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Keywords: microbial counts, whole-plant corn silage, silage deterioration.

Introduction

Elevated counts of yeasts in silages are associated with high concentrations of ethanol, and impaired aerobic stability, particularly in whole-plant or fractionated corn silage (Kung et al., 2018). Although numerous studies have associated yeast counts with fermentation profile and aerobic stability of silage, little is known about the effect of yeast populations in fresh forage prior to ensiling. The objective of this study was to assess the influence of pre-ensiling yeast populations on the nutritive value and fermentation profile of WPCS through a meta-analysis. The hypothesis of this study was that high yeast counts before ensiling would be associated with greater yeast populations in silage, which could negatively affect the fermentation profile and aerobic stability of whole-plant corn silage (WPCS).

Materials and methods

Literature published in the English language from 1990 to 2024 was searched using “corn silage yeast population”, “corn silage yeast counts”, and “whole-plant corn yeast” as keywords. The eligibility criteria included studies that (1) have presented yeast counts prior ensiling (fresh forage); (2) whole-plant corn was the silage evaluated; (3) did not use any feed source other than WPCS; (4) evaluated at least 12h of ensiling period to ensure changes on silage fermentation. After initial screening, 392 peer-reviewed articles were assessed to determine their eligibility to be included in the meta-analysis, and based on the inclusion criteria, 107 peer-reviewed manuscripts (up to 886 treatment means) were selected to analyze the effect of yeast population prior ensiling on silage nutritive value. The number of replicates, treatment means, and sources of errors were collected from tables of the published manuscripts, and data were transferred to Excel spreadsheets (Microsoft Corp., Redmond, WA) and reviewed by two trained people. Treatment means were grouped depending on the inoculant used [control (without additives), homofermentative inoculant (HOM), heterofermentative inoculant (HET), HOM + HET, chemical additive (CHE), CHE + HOM, CHE + HOM + HET]; storage length; and yeast population of whole-plant corn prior ensiling (in two groups, reaching the following ranges: 1) Low: < 6.0 log cfu/g; 2) High: > 6.0 log cfu/g. Data were analyzed using a mixed model procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC). Storage length and additive treatment group were used as continuous and categorical covariates, respectively. The model included storage temperature range as fixed effect. A random intercept was specified for each study to account for between-study variability. The covariance structure for the random effects was set to unstructured (‘type=un’). Study was considered as a random effect. The weight of each study was calculated as the inverse of the square of the variance as proposed by Roman-Garcia et al. (2016). Degrees of freedom were calculated using the Kenward-Roger option. Means were determined using the LSMEANS statement and compared using the Tukey’s test option after a significant overall treatment *F* test. Statistical significance was considered at $P \leq 0.05$.

Results

Fermentation profile, microbial counts and nutritive value of WPCS with different yeast population prior to ensiling are presented in Table 1. Dry matter concentration was 1.9 percentage-units lower for LOW compared with HIGH yeast population. Neutral detergent fiber digestibility was 1.1 percentage-units lower for HIGH than LOW yeast population. Ethanol concentration of WPCS was greater for HIGH (1.30% DM) than LOW (1.06% DM) yeast population prior to ensiling. However, 1,2-propanediol was 0.19 percentage-units greater for LOW than HIGH yeast population. Greater yeast population prior to ensiling increased yeast counts during fermentation, which was 0.32 log CFU/g greater for HIGH compared with LOW yeast population.

Table 1. The effects of yeast population prior to ensiling on nutritive value, fermentation profile and microbial counts of whole-plant corn silage

Item ²	Yeast Population ¹		SEM ²	P-value
	LOW	HIGH		
Dry matter, % as-fed	32.3	34.2	0.63	0.002
Crude protein, % DM	7.75	7.87	0.16	0.40
Soluble crude protein, % CP	50.8	55.1	2.66	0.19
Neutral detergent fiber, % DM	41.4	43.6	1.22	0.06
NDF-Digestibility, % DM	45.6	44.5	3.50	0.01
Acid detergent fiber, % DM	23.3	23.9	0.75	0.37
Starch, % DM	33.7	31.8	1.38	0.20
Dry matter losses, % DM	4.61	3.70	0.52	0.11
pH	3.81	3.86	0.43	0.33
Lactic Acid, % DM	4.99	4.92	0.22	0.76
Acetic Acid, % DM	1.54	1.44	0.09	0.34
Propionic acid, % DM	0.18	0.19	0.05	0.88
Butyric acid, % DM	0.08	0.06	0.02	0.47
Ethanol, % DM	1.06	1.30	0.11	0.01
1,2-propanediol, % DM	0.34	0.15	0.05	0.001
N-NH ₃ , % N	6.67	7.35	0.56	0.09
Lactic acid bacteria, log CFU/g	7.17	7.37	0.16	0.15
Yeast, log CFU/g	4.07	4.39	0.15	0.02
Mold, log CFU/g	2.44	1.95	0.27	0.11
Aerobic Stability, h	113	94.5	11.1	0.16

¹Low: under 6.0 log cfu/g yeast prior ensiling; High: above 6.0 log cfu/g yeast prior to ensiling.

²DM: dry matter; NDF: neutral detergent fiber.

³SEM: standard error of the mean.

Conclusions

Increased yeast population in whole-plant corn prior to ensiling increased yeast counts and ethanol concentration of corn silage. Conversely, reduced 1,2-propanediol concentration was detected. In addition, lower NDF-digestibility was observed for HIGH compared with LOW yeast population, suggesting that yeast populations in fresh forage can negatively affect fermentation profile and nutritive value of WPCS.

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Dry Matter Concentration Affects the Relationships with Variables Linked to Cassava Haylage Characteristics

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Keywords: Chemical composition, Fermentation, Multivariate approach, *Manihot esculenta* Crantz

Introduction

Haylage is a conserved feed that consists of the partial dehydration of fresh forage, followed by anaerobic fermentation, which reduces the pH and inhibits undesirable microorganisms (Muller, 2018). The aerial part of cassava (APC; *Manihot esculenta* Crantz) is an alternative feed source for ruminants, especially in tropical regions. However, APC in fresh matter contains anti-nutritional compounds (e.g., hydrocyanic acid) that can limit forage intake or cause metabolic disorders when ingested at high concentrations. The production of APC haylage can reduce the concentrations of these compounds. Dry matter (DM) content, which is directly related to the conservation and nutritional value of forage, is one of the main factors affecting haylage quality. The DM concentration modulates the fermentation profile during storage, altering the fermentation quality owing to the influence of DM on various chemical and fermentation parameters of the feed. Therefore, controlling DM content is essential for guaranteeing haylage quality. Principal component analysis (PCA) is fundamental in experiments because it reduces the dimensionality of complex data and highlights the patterns and variables that contribute the most to variability (Jolliffe and Cadima, 2016). This study aimed to investigate, using a multivariate approach, variations in the DM content of APC haylage and to identify their relationship with variables linked to the characteristics of the feed.

Materials and methods

The experiment was conducted in a field located at latitude 3°45'47" S and longitude 38°31'23" W, in the Department of Animal Science at the Federal University of Ceará. Cassava (Pretinha genotype) was harvested, chopped and dehydrated in the field until the DM contents in the ensilage reached 45, 55, 63, 68, and 73%. A completely randomized experimental design with five treatments and five replicates (25 experimental silos) was used. The haylage of cassava was stored in plastic buckets (10 L) for 65 days. The densities (DENS), gas losses (GL) and dry matter recovery (DMS) were determined. Aerobic stability (AS) was determined over 10 days in a closed environment with controlled temperature. The maximum temperature (MXT), temperature amplitude (MXA), pH at opening (pH 1), 6 (pH 6), and 10 (pH 10) days of exposure were determined. At the opening, the contents of DM, mineral matter (MM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were determined. The counts of lactic acid bacteria (LAB), sporulating bacteria (ASB), yeasts (YEA), and Molds were performed using the spread-plate method. The data were subjected to a multivariate analysis using principal component analysis (PCA) in the R® software. To identify patterns and reduce the dimensionality of the data, response variables were used as active data and DM contents as supplementary data to identify the respective correlations. A biplot graphic was generated, where the arrows represent the variables, and the direction shows the correlation between the variables. Thus, close arrows and smaller angles (close 0°) indicate a positive correlation, while larger angles (close 180°) suggest negative correlation.

Results

The results of the PCA are shown in Fig. 1. The first principal component (PC1) was responsible for 41.13% of the data variation. The DM, CP, DENS, and ADF data were the ones that contributed the most to PC1. This component represents the effect of compaction on silage characteristics, in which higher DM and fiber (NDF and ADF) contents were positively correlated with TEMP, GL, pH 1 and pH 10 contrast to higher DENS, DMR, CP, and LAB. Therefore, treatment with 73% DM resulted in high values for pH 1, NDF, ADF, and DM. These characteristics differed from those observed in the treatment with 45% DM, which showed high values of DENS, CP, DMR, and LAB. Haylages with a higher DM content had a higher content of fibrous portions, causing a dilution effect on the CP content and resulting in a negative correlation with this nutrient. It was also observed that a higher DM content resulted in higher GL and ASB counts. This probably occurred because of the lower density, which resulted in a more favorable environment for the proliferation of aerobic microorganisms, contributing to the decrease in DMR. The second component (CP2) was responsible for 22.96% of the variation in the data, where the variables pH 10, AMX TMX, and AS presented greater contributions in the formation of this component. Treatment with 55% DM presented high values of this component, which was strongly influenced by Molds and YEA counts. Similarly, MXT and MXA levels were higher and negatively correlated with AS. For this component, low values were observed for most 45% DM treatments. These results indicate that the characteristics of this treatment are related to higher AS, which has a negative correlation with other variables that contribute to the aerobic deterioration of fermented materials.

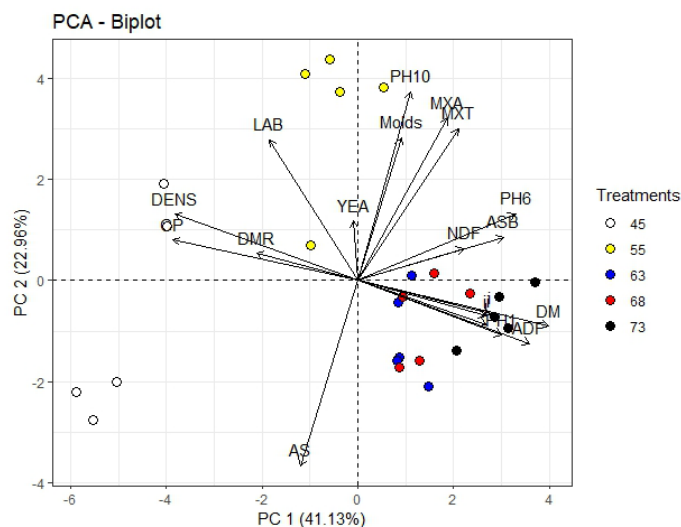


Figure 1: Principal component analysis results highlighting the variables and treatments (45, 55, 63, 68, and 73% DM) of haylage of aerial part of cassava.

Conclusions

Higher DM content was positively correlated with NDF, ADF, GL, and pH, and negatively correlated with DENS, CP, and LAB. Higher DM reduces density, favors aerobic microorganisms (ASB, Molds, and YEA), and increases GL and deterioration. Treatments with lower DM contents were positively correlated with DENS, CP, DMR, and LAB.

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Sheep Feed Preference for the Aerial part of Cassava Preserved by Different Methods

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Keywords: Silage, *Manihot esculenta* Crantz, Dry matter intake, MDS

Introduction

The aerial part of cassava (APC; *Manihot esculenta* Crantz) is another alternative feed that can be included in ruminant animal diets, particularly in tropical regions. However, the presence of anti-nutritional components, such as hydrocyanic acid, can limit its use as fresh forage. Different forms of forage conservation can be used to overcome these limitations such as silage (Kusmartono et al. 2022), haylage and hay. However, the introduction and acceptance of new feed into the diet for ruminants should be carried out with caution as animals may reject the new feed source. The preference test is a valuable tool for studying the factors that influence feed selection, preference, and intake by ruminants (Burns et al., 2001). Under controlled conditions, this method contributes significantly to the understanding of the feeding behavior of these animals, as it has the potential to measure preference and intake in the short term (Gerlach et al., 2014). Among ruminants, sheep exhibit selective feeding behavior based on sensory characteristics such as availability, texture, taste, and smell, and may develop a preference for or rejection of certain feeds. This study aimed to assess sheep preference for APC in different forms of preservation.

Materials and methods

The trial was conducted at the Federal University of Ceará, in the Department of Animal Science (latitude 3°45'47" S and longitude 38°31'23" W). The APC, genotype Pretinha, was used to produce silage (dry matter (DM) = 359.8 g kg⁻¹), haylage (DM = 533.5 g kg⁻¹) and hay (DM = 930.5 g kg⁻¹). The silage and haylage were stored for 40 days. Elephant-grass (*Pennisetum purpureum*) in natural (DM = 277.9 g kg⁻¹) was used as the standard feed. A completely randomized design was used, where each feed consisted of one treatment, making a total of four. For the preference test, 14 sheep, Morada Nova breed, male, non-castrated, aged 9 months, with an average body weight of 26.2 ± 4.24 kg, were used. The animals were kept in individual wooden pens equipped with two feeding troughs and a water fountain. The experimental period lasted 16 days, 10 days for adaptation and six days to evaluate the preference and determine the total intake (TI) of each feed. Simultaneously, each animal received two treatments separately, once a day for each day of the evaluation period. The treatments were fed at 08:00 a.m., using one trough per feed. Weight was recorded at the time of feeding (P1), after 30 min (P2) and 3 h after feeding (P3) for each treatment. The preference and total intake (TI) of the feed, in fresh and dry matter, were determined. The preference for fresh matter (PFM) and dry matter (PDM) was obtained from the difference between P1 and P2 and the TI for fresh matter (FMI) and dry matter (DMI) was calculated from the difference between P1 and P3. The preference and intake data were subjected to analysis of variance and the means were compared using the Duncan test at 5% significance, using proc GLM in the SAS statistical software. The preference for each feed was assessed in a multivariate analysis, based on a distance matrix, using the non-metric Multidimensional Scaling (MDS) technique in the R statistical software to generate the coordinate graphs.

Result

The results of animal preference and intake are shown in Table 1. Differences in feed preference and intake can differ when expressed as FM or DM. The preference for silage was higher (P<0.001) for both FM and DM than for other treatments. The PFM and PDM of elephant-grass were lower (P<0.001) than those of the other feeds. The FMI of the APC silage was higher (P<0.001) in comparison to the other feeds. The DMI was higher (P<0.001) when the sheep were fed with hay than in the other treatments. The DMI of silage and haylage were similar but higher than that of elephant-grass.

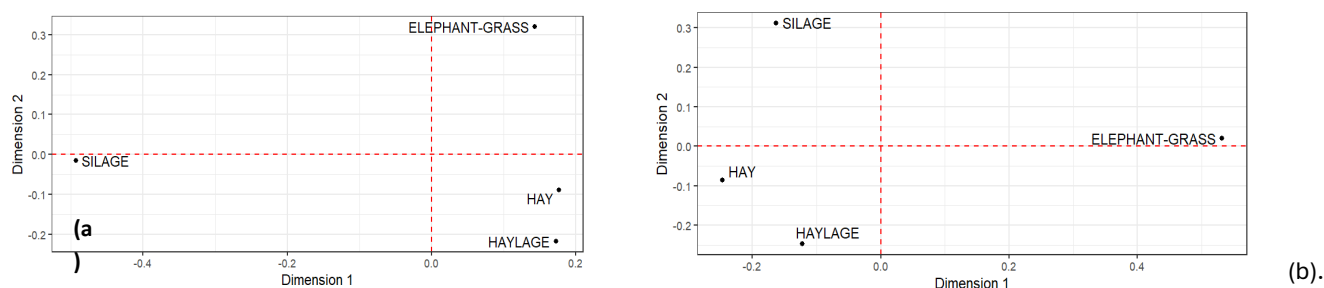
Table 1. Preference and intake of aerial part of cassava (g) in different forms of conservation and elephant-grass

	Treatment				P-value
	Silage	Haylage	Hay	Elephant-grass	
PFM	381.3a	135.64b	111.19bc	66.9c	<0.001
PDM	134.8a	73.06c	103.7b	18.59d	<0.001
FMI	575.5a	386.3b	361.8b	415.2b	<0.001
DMI	216.0b	227.7b	447.4a	150.5c	0.011

PFM: preference for fresh matter; PDM: preference for dry matter; FMI: fresh matter intake; DMI: dry matter intake.

The same letters in the line do not differ according to Duncan's test at a 5% significance level.

Figures 1a and 1b show the animals' average preferences for FM and DM, respectively. In Figure 1a, dimension 1 shows the preference for silage over elephant grass, hay, and haylage. This shows that the animals preferred silage over the other feedstuffs, as represented by a greater distance between the points. In dimension 2, the animals showed a greater preference for hay and haylage over elephant-grass. Figure 1b exhibits a lower preference for elephant-grass than for the other feedstuffs. For dimension two, silage was preferred over hay and haylage.

Figure 1. Preference of silage, haylage and hay APC by sheep in FM (a) and DM (b).

Conclusion

The preserved feed was accepted by all animals. The APC silage was preferred, suggesting that preserving forage in the form of silage is recommended for inclusion in sheep diets.

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Effect of Microbial Inoculation and Growth Stage on Ruminal Degradation Kinetics of *Glycine Max* Silage

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Keywords: commercial inoculant, isolated strain, neutral detergent fiber

Introduction

Soybean cultivated for silage production has emerged as an interesting alternative due to the higher dry matter (DM) yield (9.3–11.3 t/ha) when plants are harvested at the R5 or R6 stages (Acikgoz et al., 2007). However, the ideal maturity for ensilage needs to be determined, since the chemical composition and nutritive value of the plant alter as it matures (Oliveira et al., 2016). The use of microbial inoculants is also recommended to improve fermentation profile, reduce silage pH, and inhibit the action of undesirable microorganisms. However, *in vitro* degradability results are scarce. The objective of this study was to evaluate how maturity can interact with different inoculants to alter the ruminal degradation kinetics of soybean silage.

Materials and methods

Soybean (Intacta RR2 PRO™, Monsanto®, Goiania, GO, Brazil) was harvested from four random locations within a field located in Viçosa, Minas Gerais, Brazil. The crop was harvested at ~10 cm above ground at the R5 or R6 growth stage (i.e., 90 and 110 d after planting, respectively), and processed to particle sizes of ~1.5 cm. Subsamples of 1 kg from each location were assigned to each one of the six treatments which were a combination of three microbial inoculants [control (Ctrl; distilled water), commercial inoculant (CI; 10⁵ cfu/g soybean silage of Sil-All 4 × 4, Lallemand Animal Nutrition®, composed mainly of *L. plantarum*, *Pediococcus acidilactici*, *Enterococcus faecium*, *L. salivarius*), and isolated strain (ISO; 10⁵ cfu/g soybean silage, Strain AV14.17-*L. pentosus*, isolated previously from alfalfa silage)], and two growth stages (R5 and R6), in quadruplicate for a total of 24 mini silos. Material was individually spray-treated and hand-mixed immediately prior to ensiling in nylon-polyethylene bags (25 cm × 35 cm), with 500 g of forage added to each bag. After 60 d of storage, representative silage samples were dried at 55 °C for 72 h and ground at 1 mm. Approximately 0.5 g of composite samples for each treatment were weighed into F-57 filter bags in sextuplicate for each time point (0, 3, 6, 12, 24, 48, 72, and 96 h) to determine the DM, organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF) degradation kinetics. The zero-h bags were not incubated but were washed with other bags. Three consecutive runs were performed in Daisy^{II} incubators and used as replicates. Rumen fluid was obtained from three ruminally cannulated heifers fed a mixed diet composed of elephant grass forage and concentrate in a ratio of 80:20 with 12% of CP, on a DM basis. Animals were acclimated to this diet for 14 days before collecting ruminal fluid. After incubation times, the bags were dried at 55 °C for 72 h and subjected to analysis of DM, OM, CP, and NDF. The *in vitro* degradability of DM was estimated by the equation proposed by (Ørskov & McDonald, 1979). The degradability of NDF was estimated by the logistic model described by (Van Milgen et al., 1991). The parameters were estimated using PROC NLIN procedure (version 9.4, SAS Institute Inc., Cary, NC, USA), using the Marquardt algorithm for convergence. The NDF degradation rate was calculated based on λ , using the properties of the Γ (2) distribution. Data were analyzed as a 2 (growth stages) × 3 (microbial inoculations) factorial arrangement of treatments in a randomized block design using PROC GLIMMIX of SAS. Fixed effects included growth stages, microbial inoculation, and their two-way interaction, and the incubation run was used as a random effect.

Results

Greater DM ruminal rapidly degradable fraction (a; $P = 0.004$; Table 1) was observed for ISO (31.8%) than Ctrl (28.3%) and CI (29.5%). An interaction (MI × GS) was observed for DM ruminal slowly degradable fraction (b), where CI silage was 5.5 percentage-units greater at R6 than at R5 stage. No differences were observed for DM kd ($P > 0.05$; 4.6% h⁻¹, on average). For OM, an interaction was observed for fraction 'b' ($P = 0.01$), which for soybean silage harvested at R6 stage, CI (40.1%) was greater than Ctrl (40.1%), while ISO (35.8%) did not differ from either CI and Ctrl. At R6 stage, CI silage was 7.8 percentage-units greater than at R5 stage. No differences were observed for OM fraction 'a' and kd ($P > 0.05$; 40.0% and 4.4 h⁻¹ respectively, on average). A greater CP fraction 'a' ($P = 0.03$) was observed for silage harvested at R5 growth stage (67.6%) than when harvested at R6 growth stage (64.8%). However, soybean silage at R5 growth stage had lower ($P = 0.001$) CP fraction 'b' (22.5%) than when harvested at R6 growth stage (28.0%). No differences were observed for CP kd ($P > 0.05$; 5.8% h⁻¹, on average). For NDF ruminal degradation kinetics, fraction 'a' was greater ($P = 0.04$) for soybean silage harvested at R5 growth stage (14.0%) than at R6 stage (11.7%). A main effect of microbial inoculation was also observed for NDF fraction 'a' ($P = 0.002$), which was greater for CI (14.7%) and ISO (14.5%) than Ctrl (9.25%). No differences were observed for NDF fraction 'b' and kd ($P > 0.05$; 42.6 and 2.5% h⁻¹ respectively, on average).

Table 1. Ruminal degradation kinetics parameters of soybean silage harvested at R5 or R6 growth stages as a function of microbial inoculant¹

Item ²	Growth Stage	Microbial Inoculant			GS General Mean	SEM	P-value		
		Ctrl	CI	ISO			MI	GS	MI × GS
	R5	28.2	28.5	30.6	29.4				
a, % DM	R6	28.5	29.5	33.1	30.4	0.86	0.004	0.19	0.31
	MI General mean	28.3b	29.5b	31.8a					
	R5	36.2Aa	35.9Ba	39.1Aa	40.0				
b, % DM	R6	39.3Aa	41.4Aa	39.3Aa	37.0	0.73	0.18	0.001	0.01
	MI General mean	37.7	38.6	39.2					
	R5	34.3Aa	32.3Ba	35.3Aa	33.9				
b, % OM	R6	34.6Ab	40.1Aa	35.8Aab	36.8	1.08	0.30	0.01	0.01
	MI General mean	34.4	36.2	35.5					
	R5	9.86	16.3	15.8	14.0A				
a, % NDF	R6	8.63	13.1	13.2	11.7B	1.32	0.002	0.04	0.73
	MI General mean	9.25b	14.7a	14.5a					

¹Ctrl (distilled water), CI (CI Sil-All 4 × 4 at 10⁵ cfu/g dosage, Lallemand Animal Nutrition®, Patos de Minas, MG, Brazil), and ISO (Strain AV14.17 – *Lactiplantibacillus pentosus*, at 10⁵ cfu/g dosage). R5 and R6 of growth stage.

² a: ruminal rapidly degradable fraction; b: ruminal slowly degradable fraction; kd: degradation rate constant of fraction b; SEM: greatest standard error of the mean.

A,B,a,b Different letters within a row with lowercase and within a column with uppercase are significantly different (Tukey test, α = 0.05).

Conclusions

Inoculating soybean silage with an *L. pentosus* inoculant improved DM fraction 'a', while greater DM fraction 'b' was observed for CI silage at R6 stage. Both inoculants increased NDF fraction 'a'. Growth stage affected CP and NDF degradability fractions. There was no effect of treatment on the degradation rates of the fractions.

Acknowledgments

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Effect of Microbial Inoculation and Regrowth Age on Ruminal Degradation Kinetics of *Arachis pintoi* Silage

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Keywords: commercial inoculant, isolated strain, neutral detergent fiber

Introduction

In general, tropical legume silages are known for their low dry matter (DM) and water-soluble carbohydrates contents, and high buffering capacity, which may limit their fermentation. However, facultative heterofermentative inoculants can be used to improve the fermentation profile, while their effects on silage digestibility are still controversial. Silva et al. (2020) isolated a strain of *Lactiplantibacillus pentosus* (AV14.17) and reported its potential as an inoculant to improve alfalfa silage fermentation, but the effects on degradability have not yet been studied. Regrowth age can also alter the digestibility of legume silage due to its influence on yield and chemical composition. *Arachis pintoi* is a promising legume for silage production. According Macedo et al. (2023), its DM yield averaged 3.32 t/ha and 6.80 t/ha for 40 and 80d, respectively. The objective of this study was to evaluate how different inoculants alter the ruminal degradation kinetics of forage peanut (*Arachis pintoi*) silage at two regrowth ages.

Materials and methods

Forage peanut cv Belmonte was harvested at four random locations within a field located in Viçosa, Minas Gerais, Brazil. A uniformization cut was made at 5 cm above ground level and forage was harvest at 40 d or 80 d of regrowth age. Harvest, ensiling, sampling and silo opening, were made as detailed in the paper previously published (Macedo et al., 2023). Six treatments were used, as a combination of three microbial inoculants [control (Ctrl; distilled water); commercial inoculant (CI; 10^5 cfu/g forage peanut of Sil-All 4 × 4, Lallemand Animal Nutrition®, composed mainly of *L. plantarum*, *P. acidilactici*, *E. faecium*, *L. salivarius*), and isolated strain (ISO; 10^5 cfu/g forage peanut, strain AV14.17-*L. pentosus*, previously isolated from alfalfa silage)], and two regrowth ages (40 d and 80 d). At 60 d of storage, representative silage samples were collected and dried at 55 °C for 72 h and ground at 1 mm. Approximately 0.5 g of composite samples for each treatment were weighed into F-57 filter bags in sextuplicate for each time point (0, 3, 6, 12, 24, 48, 72, and 96 h) to determine the DM, organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF) degradation kinetics. The zero-h bags were not incubated but were washed with other bags. Three consecutive runs were performed in Daisy^{II} incubator and used as replicates. Ruminal fluid was obtained from three ruminally cannulated heifers fed a diet composed of elephant grass forage and concentrate in a ratio of 80:20 with 12% of CP, on a DM basis. Animals were acclimated to this diet for 14 days before collecting ruminal fluid. After incubation times, the bags were dried at 55 °C for 72 h and subjected to analysis of DM, OM, CP, and NDF. In vitro DM degradability was estimated by the equation proposed by (Ørskov & McDonald, 1979). FDN degradability was estimated by the logistic model described by (Van Milgen et al., 1991). Parameters were estimated using the PROC NLIN procedure (version 9.4, SAS Institute Inc., Cary, NC, USA), using the Marquardt algorithm for convergence. The NDF degradation rate was calculated based on λ , using the properties of the Γ (2) distribution. Data were analyzed as a 3 (microbial inoculations) × 2 (regrowth ages) factorial arrangement of treatments in a randomized block design (3 runs) using PROC GLIMMIX of SAS. Fixed effects included microbial inoculation, regrowth ages, and their two-way interaction, and the incubation run was used as a random effect.

Results

An interaction (MI × RA) was observed for DM ruminal rapidly degradable fraction ('a'; $P = 0.001$; Table 1), which was greater for CI (37.5%) than Ctrl (33.5%) at 80d, while ISO did not differ from either CI and Ctrl. The Ctrl silage at 40d was 4.1 percentage-units greater than Ctrl at 80d of regrowth age. The DM ruminal slowly degradable fraction ('b') and constant for degradation (kd) were not affected ($P > 0.05$; 46.3% and 4.7% h^{-1} respectively, on average). For OM ruminal degradation kinetics, an interaction was observed for fraction 'a' ($P = 0.01$), but no differences were observed after Tukey test. No differences were observed for OM fraction 'b' and kd ($P > 0.05$; 45.4% and 4.4% h^{-1} respectively, on average). Greater fraction 'a' ($P = 0.001$) of CP ruminal degradation kinetics was observed for forage peanut silage harvested at 80d (48.9%) than when harvested at 40d of regrowth age (35.4%). The CP fraction 'b' was greater ($P = 0.03$) for forage peanut silage harvested at 40d (58.3%) than when harvested at 80d of regrowth age (48.7%). No differences were observed for CP kd ($P > 0.05$; 3.2% h^{-1} , on average). For NDF degradation kinetics, an interaction was detected for fraction 'a' ($P = 0.001$). At 40d of regrowth age, the fraction 'a' was greater for CI (18.2%) than Ctrl (14.8%), while ISO (16.3%) did not differ from either CI and Ctrl. At 80d of regrowth age, the fraction 'a' was greater for ISO (22.1%) than Ctrl (15.0%), while CI (17.2%) did not differ from either ISO and Ctrl. The ISO silage at 80d was 5.8 percentage-units greater than ISO silage at 40d of regrowth age. The NDF fraction 'b' was lower ($P = 0.02$) for ISO (54.8%) than for Ctrl and CI (59.6 and 59.1%, respectively). In addition, forage peanut silage harvested at 80 d had greater ($P = 0.04$) fraction 'b' than when harvested at 40 d of regrowth age (59.3 vs 56.4%). No differences were observed for NDF kd ($P > 0.05$; 3.6% h^{-1} , on average).

Table 1. Ruminal degradation kinetics parameters of *Arachis pinto* silages harvested at 40 d and 80 d of regrowth ages as a function of microbial inoculant¹

Item ²	Regrowth Age	Microbial Inoculant			RA General Mean	SEM	P-value		
		Ctrl	CI	ISO			MI	RA	MI × RA
a, % DM	40d	37.6Aa	34.3Aa	32.9Aa	34.9				
	80d	33.5Bb	37.5Aa	36.2Aab	35.7	0.71	0.20	0.19	0.001
	MI General mean	35.5	35.9	34.6					
a, % OM	40d	41.4Aa	37.5Aa	35.7Aa	38.2				
	80d	35.7Aa	39.0Aa	39.7Aa	38.1	1.32	0.80	0.97	0.01
	MI General mean	38.5	38.3	37.7					
a, % NDF	40d	14.8Ab	18.2Aa	16.3Bab	16.4				
	80d	15.0Ab	17.2Aab	22.1Aa	18.1	0.51	0.001	0.002	0.001
	MI General mean	14.9	17.7	19.2					
b, % NDF	40d	59.6	55.9	53.7	56.4B				
	80d	59.7	62.2	55.9	59.3A	1.54	0.02	0.04	0.19
	MI General mean	59.6a	59.1a	54.8b					

¹Ctrl (distilled water), CI (CI Sil-All 4 × 4 at 10⁵ cfu/g dosage, Lallemand Animal Nutrition®, Patos de Minas, MG, Brazil), and ISO (Strain AV14.17 – *Lactiplantibacillus pentosus*, at 10⁵ cfu/g dosage). 40 and 80d of regrowth age. ²a: ruminal rapidly degradable fraction; b: ruminal slowly degradable fraction; kd: degradation rate constant of fraction b; SEM: greatest standard error of the mean. ^{A,B,a,b} Different letters within a row with lowercase and within a column with uppercase are significantly different (Tukey test, α = 0.05).

Conclusions

Delaying harvest of forage peanut increase CP solubility and NDF fraction 'b', while the commercial inoculant and the strain of *L. pentosus* AV14.17 increase NDF solubility of forage peanut silage harvested at 40d and 80d, respectively, compared to control silage. There was no effect of treatment on the degradation rates of the fractions.

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Crimping and Ensiling Pea and Faba Bean Seeds Using Different Silage Additives

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Keywords: grain legume, fermentation quality, *Pisum sativum*, *Vicia faba*, lactic acid bacteria, formic acid

Introduction

Pea (*Pisum sativum*) and faba bean (*Vicia faba*) are grain legumes that produce seeds suitable for protein and energy supplementation for both ruminant and monogastric livestock feeding. The seeds are typically stored dry, but under humid harvesting conditions, an alternative energy efficient storage method is crimping and ensiling (Jokiniemi et al., 2014). The fermentation during storage can potentially improve feed utilization by animals and degrade antinutritional factors in the seeds (Rinne et al., 2018). The objective of the current experiment was to evaluate the fermentation quality of grain legumes when crimped and ensiled using different types of additives.

Materials and methods

The grain legumes were grown in Jokioinen, Finland (60°48'N, 23°29'E). Pea (variety Eso) was combine harvested on 13 September and faba bean (variety Daisy) on 23 September in 2023. After harvesting, the seeds were crimped (crushed so that seed coat was broken,) using a farm scale roller mill (Murska VMAX, Aimo Kortteen konepaja, Ylivieska, Finland). An ensiling trial was conducted in laboratory scale using plastic vacuum bags. The additive treatments were: Control without additive (CON), a heterofermentative lactic acid bacteria inoculant (LAB; Kofasil Duo, Addcon, Bitterfeld-Wolfen, Germany at 1 g/t [2×10^5 cfu/g fresh matter]), and a blend of formic and propionic acids, and potassium sorbate (FPS; AIV Ässä Na, Eastman, Oulu, Finland at 5 l/t). Number of replicates was four. After an ensiling period of 3 months, the samples were analyzed using routine methods of Luke laboratory. The results were analyzed using PROC MIXED of SAS (SAS Inc. 2002-2012, Release 9.4; SAS Inst. Inc., Cary, NC, USA) with species, additive and their interaction as fixed effects and replicate as a random effect. The pairwise comparisons were conducted using the Tukey test with statistical significance at probability level of 5%.

Results

The composition of the seeds prior to ensiling is presented in Table 1. Peas had a lower moisture content than faba beans which can be explained by differences in crop maturity and prevailing weather conditions. The species showed typical differences in composition such as lower protein and fiber but higher starch concentrations in pea compared to faba bean. All crimped seeds showed good fermentation quality with limited protein degradation and volatile fatty acid concentrations (Table 2). The fermentation was much more intensive in faba beans than in peas as indicated by the clearly higher concentration of total fermentation products in faba beans than in peas (60 vs 9 g/kg DM). The species difference in the extent of fermentation can be attributed to the difference in the moisture content, which was higher in the ensiled faba beans than in peas (363 vs 235 g/kg). Under the conditions of the current experiments, LAB did not differ from CON in any of the parameters evaluated. Inoculants require relatively high moisture content of the material to be able to grow in the biomass, but even in faba bean with a moisture content supporting microbial fermentation, no improvements in the fermentation quality could be detected. This suggest that in the current case, the epiphytic flora was able to compete with the inoculated strains and result in a lactic acid dominated fermentation. FPS restricted fermentation of the seeds, and the species $\times$ additive interaction was significant for most fermentation quality parameters as the effect of FPS was more pronounced in the higher moisture content faba beans than in the drier peas. This trend is in line with Franco et al. (2022), who ensiled crimped barley grains ranging in moisture content from ca. 240 to ca. 350 g/kg. FPS increased the aerobic stability of peas, while for faba beans, all treatments showed good stability. Practical experience suggests that crimped ensiled seeds or grains having moisture content less than 30 % are more prone to molding and heating at feed-out than those with higher moisture content. The improvement of aerobic stability of low moisture peas can be attributed to the propionic acid and potassium sorbate included in the FPS additive.

Table 1. Composition of pea and faba bean seeds prior to ensiling.

	Pea	Faba bean
Moisture, g/kg	247	367
Chemical composition, g/kg dry matter		
Ash	41	56
Crude protein	241	287
Crude fat	16	16
Crude fiber	60	96
Water soluble carbohydrates	85	44
Starch	471	385
Neutral detergent fiber	98	133

Table 2. Composition of crimped and ensiled peas and faba beans preserved without additive (CON) or treated with a lactic acid bacteria inoculant (LAB) or an organic acid blend additive (FPS).

Species (S):	Pea			Faba bean			SEM	Statistical significance		
Additive (A):	CON	LAB	FPS	CON	LAB	FPS		S	A	S × A
Moisture, g/kg	239 ^b	239 ^b	226 ^c	367 ^a	364 ^a	359 ^a	2.2	<0.001	<0.001	0.221
pH	5.59 ^a	5.45 ^a	5.23 ^b	4.25 ^c	4.28 ^c	4.34 ^c	0.041	<0.001	0.014	<0.001
Amm. N, g/kg N	9.0 ^{cd}	10.3 ^c	7.2 ^d	40.4 ^a	39.7 ^a	35.7 ^b	0.49	<0.001	<0.001	0.031
Fermentation products, g/kg dry matter										
Ethanol	2.5 ^{ab}	1.9 ^b	0.3 ^c	3.6 ^a	4.0 ^a	3.7 ^a	0.36	<0.001	0.018	0.018
Lactic acid (LA)	4.3 ^c	4.7 ^c	0.5 ^c	53.6 ^a	47.7 ^a	35.0 ^b	2.37	<0.001	<0.001	0.023
Acetic acid (AA)	5.0 ^{bc}	5.4 ^b	3.3 ^c	10.1 ^a	10.3 ^a	10.1 ^a	0.38	<0.001	0.020	0.063
Propionic acid ¹⁾	0.10 ^b	0.11 ^b	1.43 ^a	0.11 ^b	0.10 ^b	1.23 ^a	0.019	0.124	<0.001	0.096
Butyric acid	0.05 ^a	0.04 ^a	0.05 ^a	0.05 ^a	0.05 ^a	0.05 ^a	0.003	0.022	0.297	0.904
Total ferm. prod. ²⁾	11.9 ^c	12.2 ^c	4.3 ^c	67.6 ^a	62.2 ^a	48.9 ^b	2.24	<0.001	<0.001	0.077
LA/AA ³⁾	0.87 ^c	0.87 ^c	0.14 ^c	5.31 ^a	4.72 ^{ab}	3.51 ^b	0.312	<0.001	0.003	0.265
Aerobic stability, h	137 ^b	190 ^{ab}	>241 ^a	>232 ^a	>232 ^a	227 ^a	15.2	<0.001	0.007	0.006
Fermentation loss,	12.2 ^b	11.7 ^b	7.9 ^c	20.8 ^a	20.3 ^a	20.0 ^a	0.45	<0.001	<0.001	<0.001

¹⁾ Includes the amount added in the FPS additive; ²⁾ Ethanol, lactic acid + volatile fatty acids

Conclusions

Good quality feed could be produced by crimping and ensiling of low moisture peas as well as high moisture faba beans. Moisture content greatly affected the extent of fermentation of crimped and ensiled grain legume seeds. The acid blend additive restricted fermentation particularly when high moisture faba beans were ensiled, and improved aerobic stability of low moisture peas. Under the conditions of the current experiment, ensiled seeds preserved without additive or by using a lactic acid bacteria inoculant did not differ from each other.

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Measuring Forage Quality Using On-Harvester NIRS

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Keywords: Near-Infrared Spectroscopy, Forage Quality, Precision Agriculture

Introduction

Near-infrared spectroscopy (NIRS) is a non-destructive technology that is widely used to determine nutritive properties of dried and ground forage samples in analytical laboratories. Recently, on-harvester NIRS has been gaining traction as a tool to provide producers with a real-time stream of crop quality measurements as they operate in their fields. While there is some literature evaluating the efficacy of on-harvester NIRS, most studies have been limited to testing on one platform, typically forage harvesters (Worek, 2021). This study evaluated the on-harvester performance of the Dinamica Generale EvoNIR 4.0 sensor for predicting six alfalfa properties—Moisture Content (MC), Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), Crude Protein (CP), Crude Fat (CF), and Ash—in three on-harvester configurations: self-propelled windrower (SPWR), small square baler (SSB), and pull-type forage harvester (PTFH), as well as a stationary mode for comparison. The purpose of this study was to evaluate how on-harvester NIRS forage quality measurement performs across different machinery platforms and crop conditions.

Materials and methods

A third-year alfalfa field was stage-cut into four maturities to create variable crop qualities. EvoNIR sensors were mounted on the chute of a CASE SBX540 small square baler, the swath gate of a Case WD2104 rotary disk mower, and the spout of a New Holland FP 240 forage harvester to measure crop qualities throughout the field. Over two harvests, a total of 168 samples were scanned with the on-harvester EvoNIR, collected and then scanned again with the same EvoNIR in a stationary configuration for comparison. Samples were then dried down and submitted to an analytical laboratory, where they were ground and measured for Ash, ADF, NDF, CP and CF using conventional benchtop NIRS. Sensor accuracy and precision was evaluated using R^2 , root mean square error of prediction (RMSEP), and ratio of prediction to deviation (RPD) statistics (Williams, 2014).

Results

MC was the only property predicted with significant precision ($R^2=0.89$; RPD=2.4), while ADF, NDF, CP, CF, and Ash predictions were poor ($R^2<0.54$). Sensor performance in stationary mode was comparable to on-harvester modes, except for the PTFH configuration, where predictions worsened due to residue buildup on the sensor lens.

Conclusions

The EvoNIR 4.0 sensor offers value to producers through accurate moisture measurements, but improvements are necessary for reliable prediction of other nutritive qualities. Improving self-cleaning properties in the PTFH configuration to reduce residue accumulation and building more robust sensor calibrations could improve prediction accuracy.

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Mitigating PFAS Contamination in the Conserved Forage-herbivore Livestock-human Continuum

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Keywords: PFAS, forage, milk, beef

Introduction

Per- and poly-fluoroalkyl substances (PFAS) are widely used in products including non-stick cookware, personal care products, fire extinguishing foams, food packaging materials, etc. due to their heat-resistance, as well as water- and oil-repellent properties (Sinclair et al., 2020). The widespread use of PFAS has led to their accumulation in soil, groundwater, and plants (McGarr et al., 2023). They also accumulate in the tissues of animals, including humans (Kannan et al., 2004). Exposure to PFAS has been linked with decreased fertility, low birth weight, accelerated puberty, weakened immunity and vaccine response, and hormonal balance disruption (EPA, 2024). In Maine, the CDC has set PFOS action levels at 3,400 ppt for beef and 210 ppt for milk, based on EPA water guidelines and infant milk consumption rates to protect sensitive populations (Maine CDC, 2023). In the US, one of the major sources of PFAS contamination in agricultural fields is the application of biosolids (incl. sewage sludge) to amend soil (EPA, 2024). Other PFAS sources include wastewater, landfill effluents, etc. (Ghisi et al., 2019). Livestock are exposed to PFAS through contaminated forage, water, soil, and air (Death et al., 2021). However, contaminated forage is the main source of PFAS exposure in ruminants, accounting for 78% of exposure (Brambilla et al., 2015). Our objective is to identify and develop strategies that can mitigate PFAS contamination in livestock. Our hypotheses include 1) feed binders can be used as a PFAS mitigation strategy to reduce PFAS, especially PFOS (perfluorooctane sulfonate), contamination in edible animal products and 2) animal factors such as voluntary intake, sex, and others will influence PFOS and PFOA (perfluorooctanoic acid) concentration in blood serum.

Materials and Methods

Exp.1 Assessment of feed binders to sequester forage PFAS under in vitro ruminal conditions

In the first phase of binder screening, we evaluated: clay binder 1 (CLY1) and 2 (CLY2), polysaccharide binder (PLS), carbonaceous binder (CRB), and anion exchange resin 1 (AER1), and 2 (AER2). For the second phase, binders included (AER1), cationic polymer with quaternary ammonium groups (CPQ), natural by-product feed (NBF), polysaccharide with quaternary ammonium groups (PLQ), clay binder amended with quaternary ammonium groups (CLQ) and dioctahedral phyllosilicate clay binder (CLY3). PFAS-contaminated grass (3 g) was mixed with 0.05g of each binder before adding 100 mL of rumen media. ANKOM bottles were shaken at 60 rpm for 48 h at 39°C. The percentage of PFOS binding affinity of the respective binders was calculated in relation to the control (CON). A randomized complete block design (n= 4) was used to analyze the data using SAS v.9.4. The blocks were plots within a contaminated stand. Differences were declared at $P \leq 0.05$.

Exp. 2. Identification of variables influencing perfluorooctane sulfonic acid (PFOS) and Perfluorooctanoic acid (PFOA) serum levels in mice, rats, and cattle through feature selection data mining methods

In Web-of-Science, we searched for articles measuring per- and polyfluoroalkyl substances (PFAS) in cattle and sheep. From 40 publications (for PFOS) and 44 publications (for PFOA) with specific inclusion criteria (i.e., peer-reviewed article only, domestic terrestrial mammals in 2+ publications) we extracted the following variables, where available: publication ID, sex, species, body weight, exposure duration to PFAS, treatments (animals categorized as clean animals exposed to PFAS, contaminated animals exposed to PFAS, and contaminated animals not exposed to PFAS categories), and PFOS/PFOA serum levels. The dataset was arbitrarily divided into training (70%) and test (30%) subsets, with the former used for developing the model and the latter to report the accuracy of it. LASSO and ridge algorithms were used on the training subset with a 10-fold cross-validation approach. Stepwise multiple regression was also used with AIC as the selection criterion. R 4.3.2 was used for the analysis. Differences were declared at $P \leq 0.05$.

Results

Exp. 1. First phase: Only AER1 was found to bind PFOS under ruminal conditions, with a relative binding of $52.5 \pm 8.59\%$, which was higher than any other binder tested. None of binders affected in vitro ruminal fermentation. **Second phase:** AER (up to 89.6%) and NBF (26.8%) exhibited a high ability to sequester PFOS, without affecting the in vitro ruminal fermentation.

Exp 2. PFOS: Both GLIMMIX and LASSO revealed that sex, exposure time, exposure level, and being administered PFOS were influential in determining PFOS serum levels. For LASSO model, mice, rat, and cattle differed in PFOS plasma levels. Different half-lives were reported for cattle (74 days), rats (24-83 days), and mice (30 – 43 days) (Chang et al. 2012). GLIMMIX model showed that castrated males displayed lower PFOS serum levels compared to both intact males and females.

PFOA: Both LASSO and GLIMMIX models identified exposure levels, treatment, and sex as factors influencing PFOA serum levels. Intact males displayed higher PFOA serum levels compared to both intact females and castrated males. For

the LASSO model, species type is a contributing factor with cattle showing higher PFOA serum levels than rats but lower than mice. No effect of exposure time was observed.

Non-zero mean for PFOS and PFOA plasma levels in clean animals non-exposed to PFAS suggest the presence of background environmental contamination.

Conclusions

We identified NBF and AER as promising PFAS feed binders. Both of them are safe to feed up to 3% (DM basis) in ruminant diets. For PFOA, we recommend that in vivo studies must be conducted to further investigate the influence of sex hormones and kidney function regulators on PFOA plasma levels and excretion through urine. For PFOS, we recommend conducting studies evaluating the effect of sexual hormones and the enterohepatic cycle on PFOS bioaccumulation.

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Recent Advances in Hay Spoilage and Preservation Research

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Keywords: ammonium propionate, hay spoilage, bale temperature

Introduction

Occurrence of hay spoilage increases when produced with moisture greater than 20% for small bales (~45 kg) and 16-18 for large bales (~500 kg) (Coblentz et al., 1996). Hay spoilage will result in lower nutritive value, mycotoxin contamination, and storage dry matter (DM) losses of up to 30% (Ball et al., 1998). Organic acids (e.g. propionic acid) are typically used to preserve high-moisture hay, but they are corrosive to farm equipment, require safety training to handle and. Buffering organic acids with alkali (e.g. ammonium propionate) will prevent corrosion and make handling safer. A study reported that fungi inhibiting concentration of ammonium propionate are lower than propionic acid at pH 5 and 7 (Woolford et al. (1984). The presence of ammonium cation in ammonium propionate explains its additional antifungal properties (Kwaśniewska et al., 2020). To the best of our knowledge, no published study has compared propionic acid vs ammonium propionate in the same field experiment. Therefore, this study aimed to compare the effects of organic acid-based preservative types in preserving high-moisture alfalfa hay. We hypothesize that ammonium propionate will enhance the preservation of high-moisture hay rather than propionic acid.

Materials and methods

The first experiment investigated the effects of preservative type (TY) and application rate (AR) on preserving alfalfa hay baled at high moisture ($29.36 \pm 2.70\%$). A preliminary square bale was selected from each of the 5 blocks in the field. The preliminary square bales were cut into 10 cm particles using hedge trimmers. Subsequently, the cut alfalfa hay from each preliminary square bale was divided into 7 piles, and treatment was randomly assigned to each pile for a total of 35 experimental units. The TY were 1) 67% v/v propionic acid (PRP); 2) PRP buffered with 5% v/v ammonium hydroxide (AMP); and 3) a commercial mixture of organic acids (incl. propionic and acetic acids) buffered with ammonium hydroxide (FC). All the TY were manually applied at 0, 0.25, and 0.5% AR (w/w fresh basis) standardized to a volatile organic acid equivalent (i.e. propionic plus acetic acids). The alfalfa hay was compressed into mini bales (~500 g fresh weight; $10.3 \times 10.8 \times 10.3$ cm; ~239 kg DM/m³ density) using hydraulic presses and stored for 77 d at 21°C and 70% relative humidity. After 77 d of storage, the samples were dried at 60°C until constant weight and ground to pass through 2 mm in a cyclone mill. The ruminal *in vitro* gas production kinetics of the d 77 samples were measured for 48 h using the Ankom RF gas production system. Data were analyzed as a randomized complete block design (5 blocks) with a 3 TY $\times$ 3 AR factorial arrangement using the PROC GLIMMIX of SAS v.9.4. Differences were declared significant at $P \leq 0.05$.

The second experiment investigated the effects of a buffered organic-acid preservative on large-round bales of alfalfa-timothy grass hay baled at high moisture ($23.71 \pm 2.06\%$). The treatments (TRT) were 1) FC applied at an application rate of $0.77 \pm 0.39\%$ fresh bale weight and 2) the untreated control bales (CON). Each TRT was replicated 6 times with a total of 12 large-round bales (244 kg fresh weight; 1.2×1.0 m; 220 kg DM/m³ density) produced. The experiment followed a randomized complete block design (6 blocks) with areas within the field as the blocking factor. The bales were individually weighed, placed on wooden pallets without cover, and stored inside a barn for 60 d. The internal bale temperatures were measured throughout the storage using temperature data loggers. Representative core samples (38.1 cm deep) were taken at 0, 5, 15, 30, and 60 d of storage (DAYS) and analyzed for nutritional composition and microbial populations. Data were analyzed with a 2 TRT $\times$ 5 DAYS factorial arrangement using repeated measures through the PROC GLIMMIX procedure of SAS v.9.4. Differences were declared significant at $P \leq 0.05$.

Results

In the first experiment, no differences were observed in storage DM losses for TY and AR ($\bar{x} = 9.34 \pm 3.15\%$). At d 0, the propionic acid concentration in the hay was linearly higher (0 to 0.38% w/w fresh basis) as the AR of all TY increased, but we observed no difference in d 77 ($\bar{x} = 0.10 \pm 0.01\%$ w/w fresh basis). For all TY at d 0, as the AR increased (0 to 0.5%), pH decreased linearly (6.32 to 5.72 ± 0.05), but at d 77, we observed a quadratic increase (7.25 to 7.36 ± 0.15). For all TY, 0.5% AR had lower mold counts than 0.25 and 0% (5.96 vs. $\bar{x} = 6.27 \pm 0.13$ log CFU/g fresh, respectively). The 0.5 and 0.25% AR had lower yeast counts than 0% AR ($\bar{x} = 6.20$ vs. 6.52 ± 0.13 log CFU/g fresh, respectively) for all TY. As AR increased (0 to 0.5%), the maximum bale temperature in FC decreased linearly (32.68 to $29.10 \pm 0.87^\circ\text{C}$, respectively). However, as AR increased, the maximum bale temperature decreased quadratically in PRP (32.68 to 27.54 ± 0.37) and AMP (32.67 to $27.52 \pm 0.42^\circ\text{C}$). The 0.5% AR for all TY had higher bale aerobic stability than 0.25 and 0% (41.67 vs. $\bar{x} = 2.40 \pm 13.82$ h, respectively). As the AR of all TY increased, we observed a quadratic decrease in the maximum gas production (181.34 to 174.65 ± 2.19 ml/incubated DM g). We also observed a quadratic decrease in the gas production rate as AR increases (7.75 to $7.12 \pm 0.21\%/h$) for all TY. For all TY, increasing AR from 0 to 0.5% improved the *in vitro* DM digestibility ($\bar{x} = 37.97$ to $41.27 \pm 1.29\%$, respectively).

In the second experiment, no differences in storage DM losses were observed between TRT ($\bar{x} = 2.12 \pm 1.28\%$). Across all DAYS, FC had higher DM concentration than CON (79.61 vs. $78.28 \pm 4.29\%$). The FC had a lower pH than CON (5.27 vs. 5.80 ± 0.32) across all DAYS. The NDF concentration of FC was lower than the CON across all DAYS (58.53 vs. $60.30 \pm 2.67\%$ DM basis). The FC had a higher water-soluble carbohydrate concentration than CON across all DAYS (3.16 vs. $2.63 \pm 0.16\%$ DM basis). Across all DAYS, FC decreased mold counts (5.61 vs 6.62 ± 0.91 log CFU/g fresh) and yeast counts (3.87 vs. 4.71 ± 1.73 log CFU/g fresh) than the CON. During the d 5 to d 43 of storage, the FC had a lower internal bale temperature than CON (28.06 vs. $40.92 \pm 2.93^\circ\text{C}$ for FC and CON, respectively). The maximum bale temperature was lower for FC than the CON (32.38 vs. $43.16 \pm 2.33^\circ\text{C}$) during weeks 2 to 7 of storage. Similarly, FC had lower heating-degree days than CON during weeks 2, 3, 4, and 6 of storage (26.19 vs. $83.80 \pm 17.28^\circ\text{C}$).

Conclusions

For the first experiment, applying PRP, AMP, and FC at 0.5% AR improved the preservation and digestibility of alfalfa hay produced at 29.36% moisture while reducing ruminal gas production. For the second experiment, applying FC minimized the spoilage of large-round bales of alfalfa and grass produced at 23.71% moisture by reducing nutrient losses and microbial growth during storage.

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Bacterial Diversity of Forage Sorghum Silage Treated with *Lentilactobacillus buchneri* or Organic Acids

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Keywords: Additives, lactic acid bacteria, preservation, bacterial microbiota.

Introduction

Sorghum silage is considered a valuable alternative for animal feed. However, its production poses significant challenges, such as high levels of water-soluble carbohydrates and low dry matter content at harvest. These factors contribute to excessive acidification during the initial fermentation phase, leading to substantial nutrient losses and compromising the quality and aerobic stability of the silage (Arriola et al., 2021). When silos are opened, silage is exposed to air, initiating a process of aerobic deterioration. This creates favorable conditions for the growth of undesirable microorganisms that degrade the silage (Borreani et al., 2018), thereby reducing its aerobic stability. To address these issues, the use of additives is recommended. Chemical and microbial additives have proven effective in improving silage quality by modulating the bacterial microbiota and stimulating the growth of lactic acid bacteria. Heterofermentative microbial inoculants, such as *Lentilactobacillus buchneri*, promote pH reduction and the production of organic acids, including lactic and acetic acids, the latter being essential for inhibiting spoilage microorganisms (Muck et al., 2018). Chemical additives act quickly, reducing nutrient losses and undesirable fermentative processes (Neumann et al., 2010). Our study hypothesizes that combining microbial inoculants with chemical additives during the ensiling process can modulate the bacterial diversity of sorghum silages, reducing overall diversity while maintaining a predominance of lactic acid-producing bacteria. This would enhance aerobic stability and silage quality. Therefore, the objective was to evaluate the effects of these additives, individually or in combination, on the bacterial diversity of forage sorghum silage.

Materials and methods

The experiment was conducted at the Empresa Paraibana de Pesquisa, Extensão Rural e Regularização Fundiária (EMPAER) and the Forage Laboratory at the Federal University of Paraíba (UFPB). The cultivar used was BRS Ponta Negra, and the sorghum was harvested at the dough grain stage. The experimental design was completely randomized, with four additives (AD): [Control (no additive); the commercial inoculant Magniva Steel™ based on *L. buchneri* at a concentration of 3×10^5 colony-forming units (CFU)/g (Lallemand®, Brazil), applied at 1 g/t of fresh matter (Lb); the chemical additive FreshCUT Plus™ (Kemin®, Brazil), applied at 0.5 kg/t of fresh matter (OA); and the combination of Lb and OA (Blend)]. Five replicates per treatment were used, for a total of 20 mini silos. Each laboratory silo was treated individually with spray bottles and hand-mixed immediately prior ensiling in 7.5 L plastic buckets, before being stored for 90d. At ensiling, forage samples were collected for microbiological characterization. The silos were sealed with 45 mm adhesive tape and stored at room temperature until they were opened 90 days post-ensiling. The average density was ~ 500 kg of fresh matter/m³. After opening, representative silage samples were collected to evaluate bacterial diversity. Microbial DNA was extracted from aqueous silage extracts, and sequencing was performed using the MiSeq platform (Illumina Inc., USA). The V3-V4 regions of the 16S rRNA gene were amplified using paired-end sequencing with V2 kits (2 × 250 cycles). Sequences were analyzed using the Sentinel pipeline and the phyloseq package, applying the Amplicon Sequence Variant (ASV) approach for taxonomic characterization. The non-parametric Dunn test was used for pairwise comparisons of diversity indices. Permutational Multivariate Analysis of Variance (PERMANOVA) was performed with pairwise comparisons and permutations to assess the significance of dissimilarities in distance matrices between samples and their respective groups for β -diversity analysis.

Results

The main bacterial genera identified in the relative abundance analysis were *Lactobacillus*, *Lactiplantibacillus*, *Lentilactobacillus*, *Secundilactobacillus*, and *Lacticaseibacillus*. The relative abundance results revealed distinct compositions for each treatment. The Control group displayed a diverse composition of non-lactic bacterial populations, similar to the Lb group, but with *Lentilactobacillus* as the dominant lactic acid bacterium. The OA group was dominated by *Lactiplantibacillus*, *Lacticaseibacillus*, *Secundilactobacillus*, and *Levilactobacillus*, while *Lactobacillus* was the predominant genus in the Blend group. For β -diversity, differences were observed in both metrics evaluated ($P \leq 0.05$). The Control and Lb groups had greater similarity in their bacterial composition, while the OA and Blend groups had more distinct and heterogeneous compositions (Figure 1).

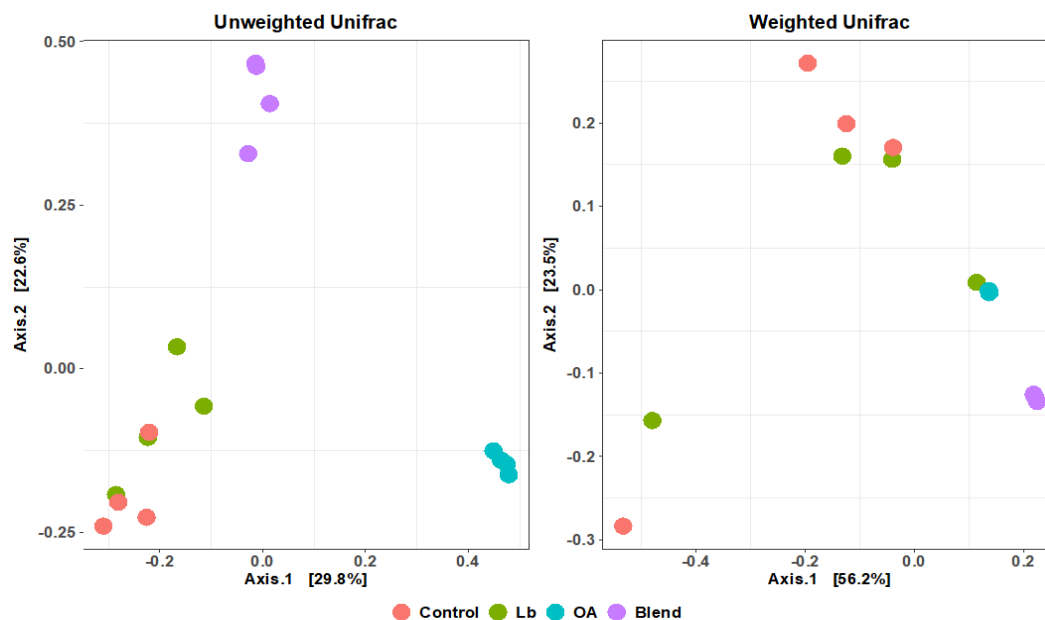


Figure 1. β -diversity analysis using principal coordinate analysis (PCoA, %) for Unweighted Unifrac and Weighted Unifrac of the bacterial community at the genus level in forage sorghum silage as influenced by additives: Control (no additive); the commercial inoculant Magniva Steel™ based on *L. buchneri* (Lallemand®, Brazil) (Lb); the chemical additive FreshCUT Plus™ (Kemin®, Brazil) (OA); and the combination of Lb and OA (Blend), at 90 days after ensiling.

Conclusions

The addition of chemical or microbial additives, applied individually or in combination, improves the quality of forage sorghum silage and contributes to modulating the bacterial community by stimulating the growth of lactic acid bacteria.

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Chemical Composition of Forage Sorghum Silage Treated with *Lentilactobacillus buchneri* or Organic Acids

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Keywords: Additives, conservation, dry matter, nutritive value.

Introduction

Sorghum silage is the second most widely used silage in Brazil, following corn silage. Additionally, the high moisture content makes increases the risk of aerobic deterioration, as it fosters the growth of aerobic microorganisms after silo opening (Arriola et al., 2021). The availability of substrates for spoilage microorganisms, combined with limited production of inhibitory compounds, worsens this issue. Chemical and microbial additives have proven effective in improving fermentation, preserving nutrients, and enhancing aerobic stability in sorghum silage (Neumann et al., 2010; Muck et al., 2018). Microbial inoculants can accelerate pH reduction in silage, promoting the production of organic acids such as lactic and acetic acids. Acetic acid is particularly important for inhibiting spoilage microorganisms (Muck et al., 2018). In contrast, chemical additives act more quickly and comprehensively, reducing undesirable processes and nutrient losses (Neumann et al., 2010). We hypothesize that combining chemical and microbial additives can enhance organic acid production and improve nutrient preservation in sorghum silage. This study aimed to evaluate the effects of these additives, either individually and in combination, on nutrient composition of forage sorghum silage.

Materials and methods

The experiment was conducted at the Empresa Paraibana de Pesquisa, Extensão Rural e Regularização Fundiária (EMPAER) and the Forage Laboratory at the Federal University of Paraíba (UFPB). The cultivar used was BRS Ponta Negra, and the sorghum was harvested at the dough grain stage. A completely randomized design was adopted in a 3 × 5 factorial arrangement, with three storage lengths (SL): 15, 30, and 90 days, and five additives (AD): control (CTRL), *Lentilactobacillus buchneri* (LB), the chemical additive FreshCUT Plus at a dose of 0.5 kg/t of fresh matter (FCUT), a combination of LB and FCUT (LB+FCUT), and FCUT at a dose of 1 kg/t of fresh matter (FCUT1), with five replicates per treatment for a total of 75 mini silos. Each laboratory silo was treated individually with spray bottles and hand-mixed immediately prior ensiling in 7.5 L plastic buckets, before being randomly assigned to be stored until reaching their assigned storage length. The average density was ~ 500 kg of fresh matter/m³. After opening, representative samples were collected for chemical composition analysis. The samples were partially dried in a forced-air oven (55°C for 72 h), ground in a knife mill with a 1 mm sieve and analyzed for dry matter (DM; method 934.01), organic matter (OM; method 972.43), and crude protein (CP; N × 6.25; method 984.13) according to AOAC (1990). Neutral detergent fiber (NDF) content was determined following the methodology of Van Soest et al. (1991). Data were analyzed using ANOVA based on the 3 × 5 factorial arrangement (three FP, five AD, and five replicates). When significant effects of FP, AD, or FP × AD interaction were detected, means were compared by Tukey's test ($\alpha = 0.05$).

Results

An interaction between FP × AD ($P = 0.001$) was observed for DM concentration. At 15 and 30 days after ensiling, silage treated with LB had greater DM concentration compared to the other treatments. However, silage treated with LB+FCUT had greater DM content than LB and FCUT1 at 90 d (Table 1). An interaction was observed for OM concentration ($P = 0.001$), which at 15d were greater for CTRL, LB, FCUT and FCUT1 than LB+FCUT. At 30d, Greater OM concentration was also observed for LB and FCUT1 than CTRL, and LB was also greater than +FCUT. At 90d, FCUT was lower compared with other treatments. An interaction was observed for CP concentration ($P = 0.001$), were at 15d was greater for LB than CTRL, FCUT, LB+FCUT. CP concentration was also greater for FCUT1 than FCUT and CTRL. Similarly, at 30 d, greater CP concentration was observed for LB, LB+FCUT, and FCUT1 than FCUT and CTRL. At 90 d, CP concentration was greater for LB+FCUT than CTRL, LB, and FCUT. In addition, FCUT1 was also greater than FCUT. Regarding the interaction observed for NDF concentration ($P = 0.001$), no differences were observed at 15 and 90 d, regardless of the additive used. However, at 30 d, the CTRL had greater NDF concentration compared to the other treatments.

Feed Quality and Conservation of Foliage from Agroforestry Through Ensiling

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Keywords: Tree foliage, agroforestry, silage, ruminants, alternative feeds

Introduction

Foliage from shrubs and trees has long been recognized as a valuable feed resource for ruminants, particularly during periods when more traditional forages are scarce or of low quality. Agroforestry, (integration of shrubs and trees into crop or animal farming) encompasses nearly 20 million ha across the EU (Mosquera-Losada et al., 2018). Apart from being feed and food sources, agroforestry systems are also carbon sinks, protect from erosion and extreme heat, and increase biodiversity. To mitigate seasonal feed shortages, conserving tree foliage through ensiling presents an innovative and sustainable approach. Tree species such as willow and poplar are particularly well suited for commercial agroforestry due to their ease of establishment, robustness, and high biomass yield. This study focuses on developing optimal preservation methods for foliage from three commonly used tree species in commercial agroforestry systems (balsam poplar, black poplar, and willow), evaluating their silage quality and nutritional profiles to enhance ruminant nutrition.

Materials and methods

In late summer (September 6, 2023), fresh foliage from black poplar (*P. deltoides* × *P. nigra*), balsam poplar (*P. maximowiczii* × *P. nigra*), and willow (*S. viminalis* × *S. schwerinii*) was manually harvested from a commercial agroforestry plantation managed by Lignovis GmbH in Großschirma/Seifersdorf (Saxony, Germany). The foliage was immediately transported to the University of Göttingen for a controlled laboratory silo experiment. Four treatments were applied to 3 kg batches of foliage from each tree species: (1) control, (2) mixture of sodium benzoate (229 g/L and sodium propionate (83 g/L Bergo® Silostabil, Bergophor GmbH, Kulmbach, Germany) applied at 4 ml/kg fresh matter (FM), (3) sugar beet molasses (621 g sugar/kg dry matter [DM]; AGRANA SUGAR GmbH, Tulln, Austria) applied at 30 g/kg FM, and (4) sugar beet molasses combined with homofermentative lactic acid bacteria (1.0×10^{11} CFU/g, Bonimal CARE, BayWa AG, Munich, Germany) applied at 3×10^8 CFU/kg FM. Ensiling was performed in double-layered, vacuum-sealed polythene bags (ROMOS; Hoedtke and Zeyner, 2011), with four replicates per treatment. The mini silos (~500 g) were stored at room temperature (15.8 ± 2.3 °C) for 91 d. The fresh foliage (d 0) and mature silages (d 91) were subsampled and analyzed for their nutrient composition including fiber fractions. Water soluble carbohydrates and buffering capacity were only analyzed before ensiling. Fermentation acid profiles, NH₃ and pH were analyzed solely in the mature silages. Data were analyzed as 3 × 4 factorial design, using the mixed procedure of SAS (Version 9.4). The model included the fixed effects of species, treatment and their interaction. Mini silo was considered as random effect. Treatment means were compared by Tukey-Kramer test. Treatment effects were declared significant at $P \leq 0.05$.

Results

Willow foliage contained more crude protein (CP; 199 g/kg DM; Table 1), neutral detergent fiber (NDFom; 441 g/kg DM) and acid detergent lignin (ADL; 177 g/kg DM), than the two poplar species. Black poplar foliage had the numerically lowest organic matter (OM) content and highest buffering capacity (45.4 g lactic acid /kg DM). Levels of water soluble carbohydrates (WSC) were similar in the two poplars (83.8 and 88.6 g/kg DM), while willow contained less WSC (61.8 g/kg DM).

Table 1. Chemical composition of the fresh foliage before ensiling

Item	Species		
	Black	Balsam	Willow
Dry matter (g/kg)	249	284	308
Organic matter, g/kg DM	869	901	916
Crude protein, g/kg DM	174	136	199
Neutral detergent fiber ¹ , g/kg DM	397	374	441
Acid detergent lignin, g/kg DM	118	105	177
Water soluble carbohydrates, g/kg DM	83.8	88.6	61.8
Buffering capacity ² , g lactic acid/kg DM	45.4	37.9	33.2

¹Neutral detergent fiber expressed without residual ash

²Expressed as g lactic acid needed to adjust 1 kg foliage DM to pH 4.0

The mean DM content of the silages (data not shown) ranged from 251 to 329 g/kg. Differences were observed among species ($P<0.001$) but there was no treatment or species $\times$ treatment effect. Organic matter was highest in willow (912 g/kg DM) and lowest in black poplar silage (857 g/kg DM; $P<0.001$). The CP content was highest in willow (206 g/kg DM) and lowest in balsam poplar silage (148 g/kg DM; $P<0.001$). Like in the fresh foliage (d 0), NDFom was highest in willow silage (495 g/kg) and lowest in balsam poplar silage (355 g/kg; $P<0.001$). Silage pH ranged from 4.5 to 5.9, while species, treatment, and species $\times$ treatment all impacted silage pH (all $P<0.001$). Within black poplar, addition of molasses (pH 4.46) as well as molasses and LAB (pH 4.52) both reduced silage pH compared to the control (pH 5.42; $P<0.05$). For balsam poplar, only the combination of molasses and LAB (pH 5.34) reduced the pH compared to the control (pH 5.67; $P<0.05$). When willow foliage was ensiled, adding molasses (pH 4.76) and molasses and LAB (pH 4.53) also reduced the pH substantially, compared to the control (pH 5.90; $P<0.001$); while the mixture of sodium benzoate and sodium propionate (pH 5.71) had no impact on pH compared the control or both molasses treatments, respectively.

Conclusions

The nutrient composition and fermentation characteristics of fresh foliage and silage from black poplar, balsam poplar, and willow demonstrates their potential as alternative feed resources for ruminants. The CP content of willow foliage is for example similar to that of alfalfa. The study also demonstrates that willow and poplar can be successfully ensiled but that the choice of ensiling technique has significant impact on the quality of silage. By incorporating tree foliage into feeding systems, farmers can potentially enhance feed availability and advance sustainable agricultural practices. Further research, addressing the feasibility of mechanical foliage harvest are under way.

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Misconceptions in Near-infrared Calibration Development: Evaluating Crop-specific vs. Mixed-crop Calibrations

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Keywords: Feed evaluation, proximate analysis, commercial laboratories

Introduction

Despite over 40 years of use in feed analysis, misconceptions regarding near-infrared reflectance spectroscopy (NIR) persist (Kern-Lubey & Husmoen, 2023). As a secondary analytical method, NIR accuracy depends on the reference data quality. Calibration evaluation is unique in that it compares predictions to unity ($y = x$), unlike most analytical methods. While some research supports crop-specific calibrations for improved accuracy (Andueza et al., 2011), others demonstrate the effectiveness of mixed-crop calibrations (Abrams et al., 1987; Kern-Lubey & Husmoen, 2023). Though accuracy may improve using the LOCAL calibrations (Berzaghi, 2000), this approach is impractical for commercial laboratories because it may select different samples across related calibrations (ex. aNDFom and NDFD) leading to inconsistent nutrient relationships. The objective was to compare performance of crop-specific versus mixed-crop calibrations.

Materials and methods

A dataset of 312 sorghum silage (SS) and 842 alfalfa silage (AS) samples was obtained (Dairyland Laboratories Inc, Arcadia, WI). Samples were submitted between May 2021 to April 2024 and analyzed for CP, ADF, and aNDFom (AOAC, 2023). Spectra were collected to predict the CP, ADF, and aNDFom using NIR calibrations developed by Dairyland. Performance of crop-specific and mixed-crop calibrations for SS (mixed small grain silage vs. SS-specific) and AS (mixed legume/grass vs. AS-specific) was compared using predictions from 50 and 300 randomly selected samples. Standard error of predictions (SEP) were compared using F-test (Snedecor & Cochran, 1989). Slopes were compared to unity ($y = x$) and between calibrations. Biases (mean of predicted – mean reference values) were compared to 0 and between calibrations. Significance was declared at $p \leq 0.05$ and tendencies when $0.05 < p \leq 0.10$.

Results and discussion

For SS, DM ranged from 17.6 to 67.9%, CP from 6.28% to 14.3% of DM, ADF from 27.4 to 46.5% of DM, and aNDFom from 40.4 to 59.9% of DM, indicating diversity in composition. Slopes for the CP predicted using mixed vs SS-specific calibrations did not differ from unity. Bias for CP from both calibrations was less than 0 ($p < 0.05$). The ADF bias for the mixed calibration tended to be higher than 0 ($p = 0.06$). Neither bias for aNDFom predictions differed from 0, but SEP for aNDFom was reduced by 45% with the SS-specific dataset (Figure 1A-C). This supports the idea that crop-specific calibrations improve accuracy, which is not consistent with previous studies in grasses (Andueza et al., 2011; Kern-Lubey & Husmoen, 2023), but these studies did not include SS, which is a C-4 grass.

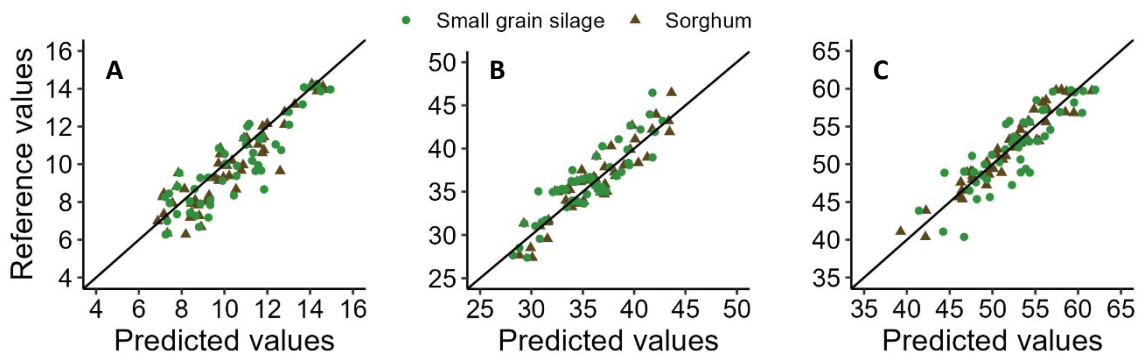


Figure 9. Predictions for sorghum silage (SS, $n = 50$) using NIR calibrations developed from mixed small grain silage ($\bullet$, SGS) and SS-specific data ($\blacktriangle$) sets for A) CP (SEP: 1.15 vs. 0.98, $p = 0.14$, slope: 0.90 vs. 0.93 ± 0.07 , $p = 0.74$, bias: -0.51 vs. -0.37 ± 0.16 , $p = 0.38$, $R^2 = 0.79$ vs. 0.83 for SGS vs. SS, respectively), B) ADF (SEP: 1.92 vs. 1.63, $p = 0.13$, slope: 0.97 vs. 0.99 ± 0.08 , $p = 0.79$, bias: 0.62 vs. 0.14 ± 0.20 , $p = 0.09$, $R^2 = 0.79$ vs. 0.83 for SGS vs. SS, respectively), and C) aNDFom (SEP: 2.54 vs. 1.40, $p < 0.0001$, slope: 0.91 vs. 0.97 ± 0.07 , $p = 0.39$, bias: -0.47 vs. 0.25 ± 0.24 , $p < 0.05$, $R^2 = 0.73$ vs. 0.92 for SGS and SS, respectively). The black line is a 1:1 bisector where reference values = predicted values.

For AS, DM ranged from 19.6 to 67.4%, CP from 15.1% to 25.0% of DM, ADF from 24.6 to 43.9% of DM, and aNDFom from 30.2 to 45.0% of DM, representing typical variation. The CP predictions using the AS-specific calibration deviated from unity ($p < 0.01$), while the bias for the CP predictions from the mixed calibration were different from 0 ($p < 0.01$). Small variations can lead to a larger deviation in the slope when the data range is narrower, as observed with CP. The SEP for CP with the AS-specific calibration was 14% lower than for the mixed calibration ($p < 0.001$), consistent with findings by Andueza et al. (2011). For ADF, neither slope differed significantly from unity, but both biases were significantly greater than 0 ($p < 0.0001$). The aNDFom SEP tended to be lower for the AS-specific calibration, although its predictions had a bias less than 0 ($p < 0.01$, Figure 2A-C). The lower SEP observed for CP with the AS-specific calibration is preferred, as it indicates improved accuracy despite a minimal systematic shift.

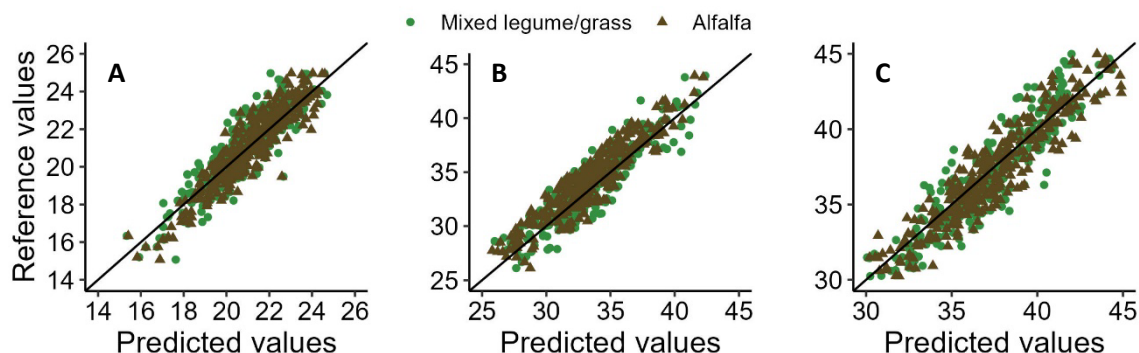


Figure 10. Predictions for alfalfa silage (AS, $n = 300$) using NIR calibrations developed from mixed legume/grass silage ($\bullet$, LG) and AS-specific ($\blacktriangle$) data sets for **A**) CP (SEP: 0.93 vs. 0.80, $p < 0.01$, slope: 1.04 vs. 1.09 ± 0.03 , $p = 0.11$, bias: 0.22 vs. -0.01 ± 0.04 , $p < 0.001$, $R^2 = 0.80$ vs. 0.85 for LG vs. AS, respectively), **B**) ADF (SEP: 1.45 vs. 1.47, $p = 0.38$, slope: 0.97 vs. 0.98 ± 0.03 , $p = 0.87$, bias: 0.36 vs. 0.77 ± 0.06 , $p < 0.001$, $R^2 = 0.82$ vs. 0.86 for LG vs. AS, respectively), and **C**) aNDFom (SEP: 1.41 vs. 1.29, $p = 0.06$, slope: 1.02 vs. 0.98 ± 0.03 , $p = 0.21$, bias: -0.13 vs. -0.27 ± 0.06 , $p = 0.10$, $R^2 = 0.83$ vs. 0.86 for LG and AS, respectively). The black line is a 1:1 bisector where $y = x$.

Conclusions

Ranges vary by nutrient and feed type, making it challenging to establish uniform acceptance criteria across all NIR calibrations. While SEP quantifies prediction error, it reflects deviations around the mean without distinguishing systematic vs. random error. The slope describes the relationship across the entire range; deviations from 1 indicate difficulty predicting extremes, but small fluctuations have a greater effect when the range is narrow. Similarly, a low R^2 can be acceptable if the range is small. Bias indicates whether predictions deviate in one direction. Including more sample variation in a calibration improves its ability to handle diverse samples but can reduce accuracy. Mixed-crop calibrations cover a broader range of feed types, which is helpful when feed type is unknown, but they are generally less accurate than crop-specific calibrations. The choice between broader applicability and higher accuracy depends on how the calibration will be used.

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Misconceptions in Near-infrared Calibration Development: Understanding Crop Year and Regional Variability

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Keywords: Feed evaluation, proximate analysis, commercial laboratories

Introduction

The perception of regional biases in near-infrared (NIR) spectroscopy likely originates from the earlier days of commercial laboratory testing, when smaller, regional labs developed NIR calibrations based on localized datasets (Mahanna, 2008). In the early adoption of NIR technology, limiting calibration datasets seemed to improve prediction accuracy (Valdes et al., 1987). However, it is now generally accepted that including diverse datasets spanning multiple crop years, regions, and hybrids enhances the reliability of NIR predictions (Shaver, 2005). Calibration models that lack such robustness may become so narrowly tailored to specific conditions that they have limited practical application. In fact, most regional labs now use licensed NIR calibrations. Yet, the perception of a regional bias remains, which may be due to, in part, cross-laboratory comparisons that fail to account for differences in reference methods or the comparison of similar but distinct nutrients (ex. aNDF vs. aNDFom). The objective of this study was to assess the accuracy of NIR calibrations for corn silage across crop years and regions in a single laboratory using consistent analytical methods.

Materials and methods

A dataset of 2,127 corn silage (CS) was obtained (Dairyland Laboratories Inc, Arcadia, WI). Samples were submitted between August 2021 to July 2024 for CS (2021 – 2023 crop years) and analyzed for aNDFom (AOAC, 2023) and starch (Hall, 2015; acetate-buffer method). Spectra were collected to predict aNDFom and starch using NIR calibrations developed by Dairyland Laboratories, Inc. The NIR calibrations were developed using a robust dataset that included samples spanning multiple crop years, hybrids, regions, and maturities, to account for variability inherent in these factors and NIR performance was assessed across crop years using 50 samples randomly selected per crop year from the Midwest and across regions using 20 samples randomly selected from Asia, Europe, the Middle East, and North and South America. Standard error of predictions (SEP) were compared using F-test (Snedecor & Cochran, 1989) for each evaluation (crop year and region). Slopes were compared to unity ($y = x$) and across the factors described for SEP. Overall biases (mean of predicted – mean reference values) were compared to 0 and as described for slope. Significance is declared at $p \leq 0.05$ and tendencies when $0.05 < p \leq 0.10$.

Results and discussion

The DM ranged from 29.7 to 43.2%, CP from 5.08 to 10.3% of DM, aNDFom from 25.9% to 50.0% of DM, and starch from 18.3% to 47.2% of DM, representing the normal range of these nutrients for CS. Slopes and SEP for aNDFom did not differ among crop year (Figure 1A), but the bias for 2021 tended to be lowest ($p = 0.08$), while the bias for 2023 was highest and also different from 0 ($p < 0.05$, Table 1). Although slope was different from 1 for 2021 starch ($p < 0.05$), slopes, SEP, and bias did not differ among crop years. Further, none of the biases deviated from 0 (Figure 1B). The limited differences in slope, SEP, and bias indicate that variation in annual factors such as growing conditions have minimal impact on the accuracy and reliability of NIR predictions.

Table 1. Comparison of validation statistics by crop year (n = 50 per year; August to July each year) and region (n = 20 per region) for corn silage

	Crop year					Region ³						
	2021	2022	2023	SE ¹	P ²	Asia	EUR	ME	NA	SA	SE ¹	P ²
<i>aNDFom</i>												
SEP	1.49	1.31	1.38	-	0.19	1.52	1.37	1.37	1.65	1.60	-	0.21
Slope	1.01	0.90	0.90	0.06	0.27	0.96	1.03	0.98	0.95	0.97	0.08	0.99
Bias	-0.50 [†]	-0.01 ^a	0.59 ^b	0.16	***	1.17	0.64	0.41	0.26	0.42	0.28	0.09
R ²	0.79	0.85	0.91	-	-	0.93	0.91	0.91	0.82	0.90	-	-
<i>Starch</i>												
SEP	1.70	1.45	1.48	-	0.13	1.22	1.58	1.51	1.61	1.31	-	0.12
Slope	0.86	0.92	0.93	0.04	0.38	0.89	1.04	0.93	0.93	1.04	0.07	0.45
Bias	-0.38	-0.19	0.13	0.19	0.26	-0.17	0.28	-0.54	0.46	-0.06	0.30	0.23
R ²	0.84	0.90	0.92	-	-	0.93	0.91	0.91	0.94	0.96	-	-

^{1,2}Lowest standard errors (SE) and P-Values (P) are presented within a comparison, *** $p \leq 0.001$

³Europe (EUR), Middle East (ME), North America (NA), South America (SA)

^{ab}Different values within a row differ within an evaluation (crop year or region, $p \leq 0.05$) or [†]tend to differ ($0.05 < p \leq 0.10$)

Slope and SEP for aNDFom did not differ among region and slopes did not deviate from 1 (Figure 1C). The bias for Asia was greater than 0 ($p < 0.05$, Table 1), but only tended to be higher for Asia than the other regions. Slopes and SEP did not differ among region for starch (Figure 1D) and none of the biases differed from 0. These results indicate minimal differences for aNDFom and starch in CS across regions, suggesting that region-specific calibrations may not be necessary. This is consistent with Abrams et al. (1987), who found that including samples from multiple regions and maturities can achieve prediction accuracy comparable to equations developed for specific regions, particularly in hay.

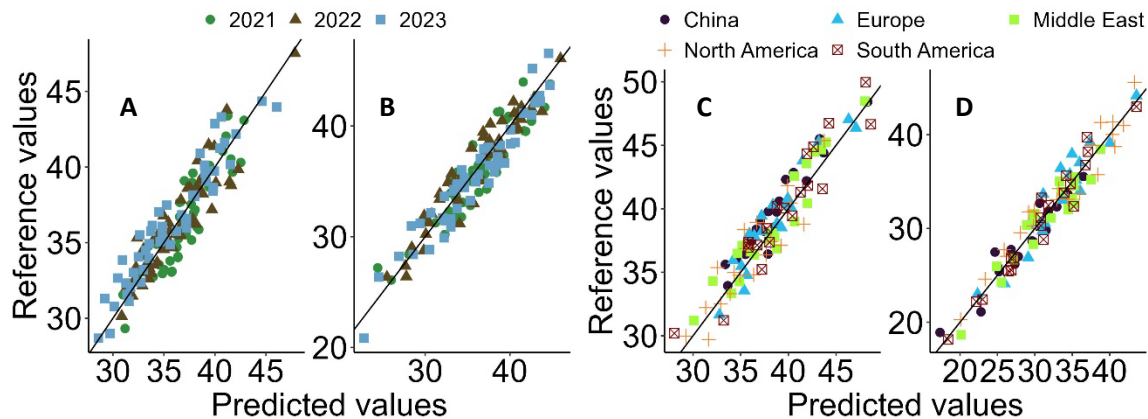


Figure 11. NIR predictions for corn silage over three crop years (August – July each year) for **A)** aNDFom (%DM) and **B)** starch (%DM) and five regions for **C)** aNDFom (%DM) and **D)** starch (%DM). The black line is a 1:1 bisector where reference values = predicted values.

Conclusions

Error of NIR predictions did not vary significantly across crop years or regions when using diverse datasets to develop robust NIR calibrations for CS. Collecting CS over time from the same area captures variation in growing conditions, comparable to sampling from different regions, and it is unlikely that spectral characteristics differ across these factors. Regional variation in NIR results is more likely to stem from differences in reference methods, rather than errors due to the NIR calibration.

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Investigation on Trypsin Inhibitor Activity Reduction During Ensiling of Freshly Harvested Soybeans with High-moisture Corn

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Keywords: Soybeans, high-moisture corn, co-ensiling, silage additives, trypsin inhibitor activity

Introduction

Locally grown soybeans are a high-quality feed with a favorable amino acid profile and offer agronomic benefits. Since they are harvested at a high grain moisture content, drying is necessary to prevent spoilage. Furthermore, prior to being used as feed for pigs and poultry, soybeans require thermal processing (toasting) to degrade antinutritional compounds such as trypsin inhibitors. Both processes are energy- and labor-intensive. Furthermore, temperature and treatment duration must be optimized during processing to achieve the best possible inactivation of the inhibitors, without risking over-heating, which could lead to protein damage. Ensiling of ground high-moisture corn, which is harvested around the same period as soybeans, however, is an established practice in Germany. Through the joint ensiling of soybeans with corn grain, the favorable ensiling characteristics of corn grain could be used to ensile the more difficult-to-ferment beans. The objective of this study was, therefore, to examine the fermentation quality of soybean-high-moisture corn mixtures and the extent of trypsin inhibitor activity (TIA) reduction during the co-ensiling process.

Materials and methods

Freshly harvested ground high-moisture corn (S0) and soybeans (S100), as well as their mixtures consisting of 15 % (S15), 30 % (S30), and 45 % (S45) soybeans, were ensiled in a laboratory silo test in a two-factorial experimental design in three replicates for 90 days in mason jars according to the DLG Testing Guidelines (DLG, 2018; Pauly & Wyss, 2018). In addition to the untreated control, the effect of a biological ensiling agent with DLG action category 1 (improvement of the fermentation process: *L. plantarum*) and a biological ensiling agent with DLG action categories 1 & 2 (improvement of the fermentation process & increase in aerobic stability: *P. pentosaceus*, *L. brevis*, *L. buchneri*, *L. plantarum*, *L. rhamnosus*) were tested in all mixtures. The effect of a chemical silage additive (propionic acid: 5 l/t) was additionally examined in S15. The analysis of TIA was conducted according to EN ISO 14902:2001, with a detection limit of 0.5 mg/g. Results were analyzed by ANOVA using the GLM procedure of SAS 9.4, considering the effects of the mixture (S0-S100) and treatment (control, silage additives).

Results and discussion

All variants were successfully preserved over a period of 90 days. Regardless of the soybean proportion, no signs of undesirable fermentation or mold growth were observed. Silages containing up to 30 % soybeans achieved a pH below 5.0. Above this threshold, preservation was ensured through high dry matter content and the exclusion of oxygen. Butyric acid could not be detected in any of the silages. Therefore, as the proportion of soybeans increased, resulting in higher dry matter content, a decrease in fermentation intensity was observed (Table 1). All mixtures containing soybeans demonstrated high aerobic stability, whereas pure high-moisture corn heated up rapidly under exposure to air. TIA in the fresh material was below the detection limit for high-moisture corn and 42 mg/g DM for soybeans with corresponding gradations for the mixtures of 7.4, 14.4 and 21.0 mg/g DM for S15, S30 and S45 respectively. TIA was reduced during the ensiling process (Table 1). The relative TIA reduction during ensiling decreased with increasing soybean proportion and thus decreasing fermentation intensity: 56 % (S15), 39 % (S30), 38 % (S45), and 8 % (S100). In absolute terms, however, the degradation rate increased up to a soybean proportion of 45 %: While 4.2±0.1 mg TIA/g DM were degraded on average in S15, it was 5.7±0.5 mg/kg DM in S30 and 8.0±0.9 mg/kg DM in S45. In S100, TIA reduced by 3.4±2.1 mg/kg DM, with a high variance. Gao et al. (2013) report a higher degradation of TIA in soybean meal with the addition of *Lactobacillus brevis* and *Aspergillus oryzae* during solid-state fermentation, with reductions of 57 % and 89 %, respectively, to 6.4 and 1.6 mg/g DM. It should be noted that the soybean meal was moistened to 53 % and 39 % DM, which provided much more favorable growth conditions for the microorganisms compared to the present study. The application of silage additives, which were applied outside the optimal dry matter range, had no significant effect on fermentation dynamics, aerobic stability, fermentation quality or TIA reduction.

Table 1: Fermentation quality after 90 days, aerobic stability, dry matter loss and trypsin inhibitor activity of untreated control silages (N=3) made from high-moisture corn (S0), soybeans (S100), and their mixtures (S15, S30, and S45: 15, 30, and 45 % soybeans, respectively).

	DM g/kg	pH 3 days	pH 90 days	Lactic g/kg DM	Acetic	Ethanol	NH ₃ -N % TN	DM %	AE days	TIA mg/kg DM
S0	688	4.22	4.18	15	2	2	5	3.4	3.5	0
S15	710	4.92	4.26	17	3	2	3	3.2	10.9	3.4
S30	732	5.58	4.49	17	4	1	2	3.2	10.9	8.8
S45	756	6.15	5.19	5	3	0	1	2.9	10.9	13.7
S100	849	6.58	5.87	0	0	0	0	2.5	10.9	37.0
SEM	2.4	0.146	0.018	1.4	0.2	0.2	0.4	0.01	0.06	0.79
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

DM: dry matter, TN: total nitrogen, AE: aerobic stability (after 49 days of ensiling and two periods of air exposure for 24 h), TIA: trypsin inhibitor activity

As an additional observation, it is worth noting that silages made from corn-soybean mixtures showed a tendency to form strong agglomerations, which may pose challenges during feed out phase in practice. In contrast, pure corn and pure soybean silages could be removed from the mason jars without difficulty. Furthermore, a noticeable browning was observed in the mixtures, with the intensity of the browning increasing as the soybean content rose.

Conclusions

Ensiling soybeans together with high-moisture corn may represent an innovative approach to reduce the need for drying and/or toasting the soybeans. In this laboratory silo trial, high-moisture corn, soybeans, and their mixtures containing 15 %, 30 %, and 45 % soybeans were successfully preserved over 90 days without undesirable fermentation, mold formation, or spoilage, while also demonstrating high aerobic stability in the soybean variants. However, the high dry matter content of the soybeans limits the fermentation process, resulting in a weaker pH reduction as the soybean proportion increases. It remains to be evaluated whether the observed TIA reduction, which correlated with the pH drop, is sufficient to enable the effective use of the respective corn and soybean mixtures in pig and poultry diets. Further research should be conducted to assess the feed value of the silages, the impact of the ensiling process on additional antinutritional factors such as lectins, aspects of fat quality (e.g., oxidation), and practical considerations related to process design, including harvesting, storage, removal, and feeding.

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Silage Diagnostics –A Systematic Approach Towards Troubleshooting and Future Preventive Action

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Keywords: aerobic stability, chemical analysis, hygienic status, measurements, sensory evaluation

Introduction

Performance depression and loss of vitality in cattle herds often result from feed deficiencies. Moist-conserved forages constitute a major part of the feed of farm ruminants and are more prone to spoilage than dry feed concentrates. Thus, a systematic feed check in the daily working routine and in the event of problems is crucial for prevention and troubleshooting. The steps from addressing a silo to laboratory analyses of silage are presented and explained using five approaches leading to a holistic silage quality evaluation. The focus is on all the aspects besides the classical nutritional composition of a feed. This holistic perspective enables the correct use, management, and handling of the silage. It is an update of the silage evaluation systems, which have evolved historically (Martens & Löffler, 2025).

Materials and methods

These are the recommended parts of the silage assessment system to determine silage quality (Fig. 1):

- I. Approaching the silo where the silage is stored and inspecting it from all sides. Examining the silo cover for any damages, determine the degree of compaction, the impact of the removal technique on the silo face, and temperature (for details, see Maack et al., 2025).
- II. Take silage samples and evaluate their dry matter, odor, color, and structure (see Resch et al., 2025).
- III. Signs of aerobic spoilage should be monitored regularly. Temperature and pH changes, as well as sensory evaluation, serve as valuable indicators (for details, see Martens & Resch, 2025).
- IV. Taking representative samples of the silage, mixing them, and sending a sample to the laboratory in accordance with good practice for analyzing and evaluating parameters of fermentation and nutritional quality (for details see Schneider et al., 2025).
- V. If hygienic issues are suspected but not obvious, samples should be taken aseptically for microbiological analyses (for details, see Wagner et al., 2025).

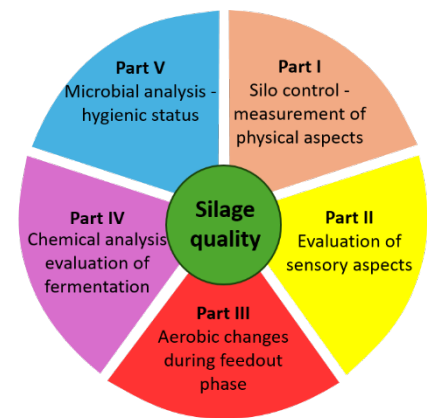


Figure 1. Five perspectives for evaluating silage quality: The silage assessment system.

Results and discussion

Once these steps have been completed, the single results must be ranked and weighted. A normalized radar chart can serve as a tool to identify the silage's strengths and weaknesses and to deduce its best use on the farm and management recommendations (Fig. 2 a & b are examples).

The presented chart (Fig. 2 a) shows, for example, a maize silage, which has deficiencies in compaction, which resulted partly from insufficient chopping. This was assessed by silo face control (I). Furthermore, temperature, condensed water below the silage film, and pH increase indicate aerobic instability (I, III, IV). An alcoholic smell points to the activity of yeasts (II), which is confirmed by chemical and microbiological analyses (V). Recommendations: An enhanced feed-out rate is one immediate measure. The chop length and compaction should be carefully controlled for the next ensiling season. The farmer should consider an ensiling agent to enhance aerobic stability. The second chart (Fig. 2 b) shows a grass silage, which has a relatively high pH value about the low dry matter content (I, II, IV). Traces of butyric acid are perceptible. It has a dark color; the leaf structure is smeary. Dirt remains on the hands (II) after handling the silage. The ammonia content (IV) confirms proteolytic processes, and the microbiological analysis shows clostridial spores (V).

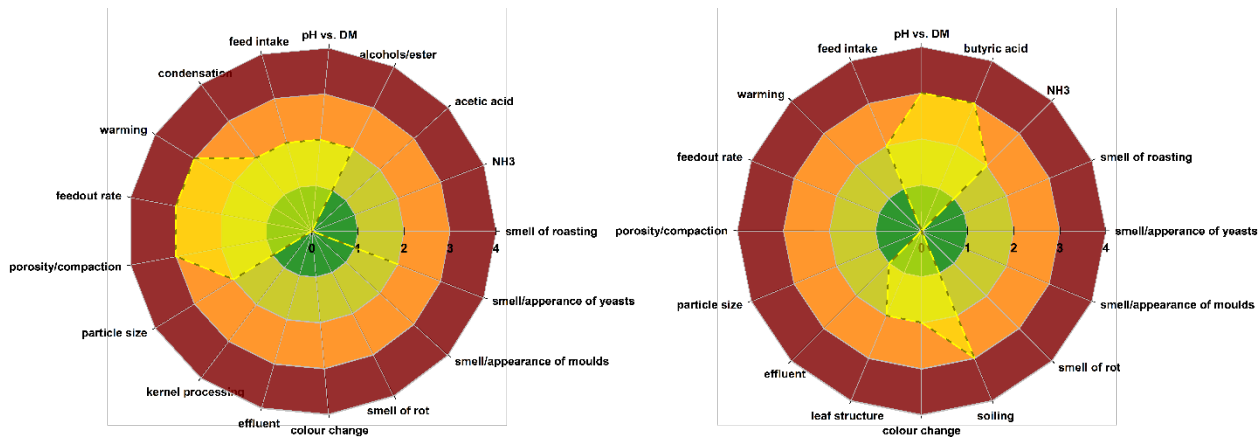


Figure 2. Outcome of a differentiated silage evaluation; example a) maize silage, b) grass silage. The problem severity increases with the distance from the center to the outer ring (scale 0-4).

Recommendations: The lower energy content has to be compensated in the ruminant ration. Furthermore, feed with hygienic deficiencies should be fed in the less sensitive phases of a cattle's lifecycle. For the next harvest season, cutting at ideally dry weather conditions and rapid wilting to >30% DM should be aimed for. An ensiling agent for difficult ensiling conditions should be considered.

Thus, the findings will help identify shortcomings in the ensiling process and estimate the current use value in the feed supply for livestock.

Conclusion

Understanding the connections between the processes in the field and the final feed quality shall lead to planning for the following growing season. Applying the five suggested parts of the silage assessment system will help to characterize a silage and offer the opportunity to identify its problems for consequent troubleshooting.

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Silage Troubleshooting – Part IV: Evaluation of Fermentation Quality Based on Chemical Results

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Keywords: Roughage, pH, fermentation acid profile, dry matter content, DLG key

Introduction

The quality of roughage is a crucial factor for a farm's success. Fermentation quality plays a crucial role, as it can influence feed intake and performance parameters (Steen et al., 1998). Moreover, fermentation quality is closely related to the extent of silage losses. To identify potential optimization in silage management, silage samples should be analyzed in a laboratory for fermentation quality. In this context, a simple classification of the results is important. In German-speaking regions of Europe, the German Agricultural Society (DLG) key for assessing the fermentation quality of ensiled forage based on the chemical analysis (Part B; DLG, 2006) is a commonly used tool. It is used, for instance, in feed laboratories to evaluate analysis results. This evaluation considers the butyric and acetic acid content as well as the pH level depending on the dry matter (DM) content and provides an overall score to help farmers interpret results. The scoring key is outlined below as one part of a systematic approach of silage diagnostics, the silage assessment system, towards troubleshooting, and future preventive action (Martens et al., 2025).

Procedure of evaluation

The DLG (2006) evaluation key assesses the contents of butyric acid (BA) and the sum of acetic and propionic acid (AA) with deductions of points when concentrations are above 3 g/kg DM (BA) or 30 g/kg DM (AA) respectively. Additionally, the pH value is scored, depending on the dry matter content, which is divided into three ranges (<30 %, >30-45 %, and >45 % DM). The corresponding points can be read from tables or calculated using provided regression equations, summarized in Table 1. Points are assigned based on the concentration of the mentioned fermentation acids and the pH value, resulting in a score categorizing the silage as very good, good, needing improvement, poor, or very poor. The assessment applies to not moldy, contaminated, or spoiled silages.

Discussion

The chemical analysis of fermentation quality is an important, objective tool for evaluating silage. The DLG key simplifies the results into a format that is easy for farmers to understand, thereby increasing the rate of practical implementation. While the key was initially introduced on a trial basis, it has been in use for almost two decades, indicating its practical value. It complements sensory evaluations of silage (see part II, Resch et al. 2025, this volume) in daily operations by allowing farmers to calibrate their own senses against objective measures. Additionally, it complements troubleshooting sections provided by parts I, III, and V (Maack et al., 2025; Martens & Resch, 2025; Wagner et al., 2025; all in this volume). The evaluation key broadly applies to all types of forage silages, regardless of ensiling methods or DM content. Moreover, it requires only a few parameters (DM, pH, AA, and BA), making it a practical approach that avoids needing more complex analyses while providing a comprehensive assessment.

In terms of protein supply for livestock, preserving protein quality is gaining increasing importance in the context of sustainability. The extent of protein degradation in the DLG key is indicated solely by the BA content, which is not a precise indicator, as protein degradation can occur to a significant extent without any detectable butyric acid (Kofahl, 2008). NH₃-N is a more direct degradation product of protein, but its significance is also limited (McDonald et al., 1991) and is influenced by the composition of the silage material. In this context, the protein fractions could be considered. When interpreting acetic acid levels, it is important to consider that concentrations above 30 g/kg DM may also be attributable to the activity of heterofermentative lactic acid bacteria and do not necessarily indicate faulty fermentation (Weiß & Krause, 2011). In addition, the extent of aerobic instability cannot be readily inferred from laboratory analysis, representing one of the biggest challenges in agricultural practice. Further consideration should be given to alcohol and esters. If increased alcoholic fermentation by yeasts occurs during ensiling, leading to elevated alcohol levels, this can result in the formation of esters and other volatile organic compounds (VOC). Silages containing this VOC may smell like acetone, and feed intake can be severely impaired. The evaluation could also be enhanced by considering the total acid content, as high acid levels can reduce palatability and feed intake, thus affecting animal performance. When adjusting the silage process, the parameters of soil contamination and residual sugar content are also significant factors to consider. The addition of further parameters would increase the informative value, but also the analytical effort and would lead to a more complex evaluation.

Table 1: Calculation of the DLG key for assessing the fermentation quality of ensiled forage based on the chemical analysis (Part B; DLG, 2006) considering the contents of butyric and acetic acid as well as the pH value depending on the dry matter content.

Evaluation criterion (x)	Score/regression equation (points)	Range limitation
Butyric acid (%) [†]	Score=-29.985*ln(x)+53.982	0...90
Acetic acid (%) [‡]	Score=-59.158*ln(x)+63.256	0...70
pH-Value		
Dry matter ≤ 30 %	pH ≤ 4.0: 10 points, pH >4.0-4.6: score=186.9+4,827x ² -63.645x, pH ≥4.6: -5	
Dry matter >30-45 %	pH ≤ 4.5: 10 points, pH >4.5-4.8: score=658.2+23,81x ² -251.19x, pH ≥4.8: 0	
Dry matter >45 %	pH ≤ 5.0: 10 points, pH >5.0-5.3: score=789.76+23,81x ² -275.0x, pH ≥5.3: 0	
Total score	The sum of butyric acid, acetic acid, and pH points: 100...90 = grade 1 (excellent), 89...72 = grade 2 (good), 71...52 = grade 3 (in need of improvement), 51...30 = grade 4 (poor), <30 = grade 5 (very poor)	0...100

[†] Butyric acid content is the sum of i-butyric acid, n-butyric acid, i-valeric acid, n-valeric acid, and n-caproic acid.

[‡] Acetic acid plus propionic acid.

Conclusion

The DLG (2006) evaluation key is a practical tool for assessing silage fermentation quality based on a few parameters. It has proven useful for nearly two decades in helping farmers understand and optimize silage quality. The addition of the fermentation quality assessment key should focus on ethanol content and continue to address the assessment of aerobic stability and protein quality aspects.

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Silage Troubleshooting – Part V: Classifying the Hygienic Status of Silage

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Presented by **Mariana Schneider**

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Keywords: mesophilic aerobic bacteria, molds, orientation and reference values, sulfite-reducing clostridia, yeasts

Introduction

Since the 1990s, the Feed Microbiology Working Group of the Association of German Agricultural Inspection and Research Institutes (VDLUFA) has been developing orientation values for the microbiological quality assessment of a large number of feed types. In principle, several hundred unsuspecting samples are used for this purpose, which have been processed by the participating laboratories (from Germany, Austria, and Switzerland) in their routine analysis over the years. The colony counts determined in this process are divided into seven microbial groups based on 19 typical indicator organisms representing product-typical/field-based or spoilage-indicating aerobic mesophilic bacteria, molds, and yeasts. After a technical and statistical evaluation, orientation values are determined and published on the VDLUFA homepage. This also includes data for silage. Guideline values for sulfite-reducing clostridia were also determined comparably.

Furthermore, there is a molecular biological method (qPCR) for quantifying *Clostridium* spp. in grass-silage (Dorn-In et al., 2023). Silage spoilage is mainly caused by microorganisms. The aim of this work is to present how to evaluate and classify silages correctly in terms of their microbial colonization and hence their potential hygienic risks. The presented scheme is part of the silage assessment system (Martens et al., this volume).

Materials and methods

The colony counts of aerobic mesophilic bacteria, molds, and yeasts are determined using VDLUFA method 28.1.2 (Tryptose agar, 30 °C for 4d, RoseBengal agar, and DG18 agar, 25 °C for 5d) and divided into seven microbial groups (VDLUFA 28.1.3, header of Table 1). The exact application of these orientation values (VDLUFA 28.1.4) is carried out according to the following logic: Four quality levels are from I – normal to IV - spoiled. If all seven orientation values are undercut, this is referred to as quality level I. Quality level IV is achieved if at least one orientation value is exceeded 10 times.

The colony counts of vegetative sulfite-reducing clostridia were determined using VDLUFA method 28.3.2 (TSC agar, 37 °C, anaerobic, 1-2d). A comparable procedure was applied to develop reference values, but only for grass silage, maize silage, and total mixed rations. There is no 10-fold tolerance for the reference values.

The qPCR targeting the 16S rRNA gene of *Clostridium* spp. was tested for inclusivity and exclusivity in silico using the 16S rRNA sequences of 350 strains of microorganisms available in GenBank (NCBI). Subsequently, the specificity of qPCR was tested with DNA extracts of *Clostridium* spp. (n=40) and other bacteria (n=37). The standard for quantifying clostridia in grass silage was *C. perfringens* (DSM 756) with a concentration of 10² to 10⁶ CFU/g. The ISOLATE II Genomic DNA-Kit (Bioline/Meridian) was used to extract bacterial DNA from silage samples.

Results

In 2004-2008, 5 laboratories analyzed n=386 grass silages, which were used to derive orientation values (Table 1). In the case of maize silage, 10 laboratories analyzed n=449 samples from the years 1999-2008 (Table 1).

Table 1. Extract from Table 3 of the orientation value scheme. Microbial groups (MG) 1 to 7. MG 1: product-specific/field-grown native bacteria: Yellow pigmented bacteria, Pseudomonas/Enterobacteriaceae, coryneform bacteria, etc.; MG 2: spoilage indicating bacteria: *Bacillus*, *Micrococcus*, *Staphylococcus*; MG 3: spoilage indicating bacteria: Streptomyces; MG 4: product-specific/field-grown native molds and Dematiaceae: *Acremonium*, *Verticillium*, *Fusarium*, *Aureobasidium*, *Trichothecium*, *Ustilago*, etc.; MG 5: spoilage indicating molds: *Aspergillus*, *Penicillium*, *Scopulariopsis*, *Wallemia*, *Paecilomyces*, etc; MG 6: spoilage indicating molds: *Mucorales*.

Fermented feed ¹⁾	Mesophilic aerobic bacteria x 10 ⁶ CFU/g			Molds and Dematiaceae x 10 ³ CFU/g			Yeasts x 10 ³ CFU/g
MG	1	2	3	4	5	6	7
Maize silages ²⁾	0.4	0.2	0.03	5	5	5	1000
Grass silages ^{2),3)}	0.2	0.2	0.01	5	5	5	200

CFU colony forming units

1) applies to silages after some weeks of anaerobic storage and not to freshly ensiled feeds (VDLUFA 28.1.4, 4.2.3)

2) Microbial groups 1 and 4: Microorganisms originate from the field and are thus native to the plant material before ensiling; they die under optimal ensiling conditions.

3) Ensiling products, mainly consisting of gramineae species.

For grass silage, four laboratories analyzed n=381 samples between 2010 and 2016, setting a Clostridia reference value of 500 CFU/g. The reference value for maize silage is 200 CFU/g and was derived from n=162 samples, the same number of laboratories, and the same time period.

A total of 90 grass silage samples from the years 2020-2021 were subjected to qPCR. The result is convincing; the clostridia were found between 1.4×10^3 and 1.5×10^5 CFU/g silage. However, the values obtained with this qPCR cannot be compared with the VDLUFA orientation values, as the qPCR quantifies not only sulfite-reducing Clostridia but all species of the genus *Clostridium*.

Conclusions

The available data is based on the range of microorganisms and the frequency of colony counts that occur in the analyzed silages. Extending the database to other parts of the world, especially other climatic zones, would be interesting. The use of orientation or reference values is an important benchmark for assessing the microbiological quality of silage or feed samples in general. This can be important, for example, to prove compliance with legal requirements, clarify the suitability of feeding for livestock, or determine the cause of problems in an animal population. Animal health should not be directly linked due to the involvement of many other factors. This would require further investigation of the microorganisms to determine whether pathogens are present in individual cases.

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Effects of Inoculated Corn Silage on Fermentation, Aerobic Stability, and Feed Preference in Holstein Heifers

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Keywords: Silage inoculant, Feeding preference, Aerobic Stability

Introduction

In addition to nutrient conservation, the importance of ensiling extends to improving the stability of forages during aerobic feed-out. Instability during aerobic exposure not only contributes to additional dry matter losses, reductions in nutritional value, and increases the growth of undesirable microorganisms, but can also impact the organoleptic aspects (Borreani et al., 2018, Kung et al., 2018). Previous work has demonstrated the impact of fermentation profiles and resulting organoleptic aspects on feed intake (Bandla et al., 2024). For instance, poorly fermented and deteriorating silage often produce undesirable end products which negatively affect dry matter intake and feeding behavior in animals. The application of silage inoculants containing a mixture of homofermentative and heterofermentative lactic acid bacteria (LAB) is an important practice for improving aerobic stability along with good silage management practices (Muck, 2018). However, few studies have evaluated the impact of inoculated silage on dry matter intake as a proxy for palatability. Therefore, the objectives of this study were to characterize the impact of inoculation on silage quality at desiling and subsequent dry matter intake either at the time of desiling or after 24 hours of oxygen exposure.

Materials and methods

A conventional corn hybrid was harvested at the William H. Miner Agricultural Research Institute (Chazy, NY, USA) on 2023-10-02 and either untreated (Con) or treated (Tx) with a multi-strain inoculant containing homofermentative strain *Pediococcus pentosaceus*, and two heterofermentative strains *Lentilactobacillus buchneri* and *Lentilactobacillus hilgardii* at 2.5×10^5 cfu/g forage (Lallemand Specialties Inc., Milwaukee, WI, USA). Material was ensiled in bunkers for approximately 187 days. Bunker samples were collected relative to the experimental feeding period on days 0, 6, and 12. After defacing bunkers for feeding, the defacer was used to remove silage at 50 and 150 cm below the surface onto a clean tarp. Following removal from each height, the piles were subdivided into five. Each of the five piles per height were thoroughly mixed before collecting subsamples. Subsamples were collected for dry matter (DM) determination, microbial enumeration of LAB and yeasts/ molds, and for a 10-day aerobic stability assay. Finally, samples were composited across depth for wet chemistry analysis of the fermentation profiles and nutritional aspects.

A total of 16 pregnant Holstein heifers (16-21 months of age) were enrolled for the feeding trial and adapted to tiestalls for seven days prior to the experimental period. Approximately 45-60 cm of corn silage was removed from each bunk daily and placed into forage carts. Forage carts were stored out of direct sunlight in an open area at ambient temperature. Heifers were introduced to one of four treatments for 3 hours daily for the first 4 days : freshly defaced Con silage (C0), Con following 24 hours of aerobic exposure (C24) in the forage cart, freshly defaced Tx silage (T0), and Tx following 24 hours of aerobic exposure (T24) in the forage cart. After 3 hours, the bins were removed and a TMR was fed *ad libitum* for the remainder of the day.

During the 12-day treatment period, palatability of silage treatments was evaluated by offering each heifer two bins of different silage treatments for 3 hours. Intake was measured after 30 minutes and 3 hours. All silage treatments were sampled daily and dried in a forced-air oven at 105°C for 18 to 24 h for DM determination. Dry matter intake was determined for each heifer after 30 minutes and 3 hours of offering silage.

All data were analyzed in R Studio (R Version 4.4.2). For the ensiling data, normality was evaluated through Shapiro-Wilk test and visualized with Q-Q plots. All bunker data were analyzed through Kruskal-Wallis analysis for parameters at individual timepoints. Relative palatabilities of the four feed types were estimated via a paired comparisons analysis as described in Pape et al. (2024). This method relies on the assumption that the more a feed is preferred, the greater the intake of that feed will be as percentage of the total intake of the pair of simultaneously-offered feeds. We applied a range of thresholds (50%, 60%, 70%, and 80%) to the intake percentage above which one feed was designated as preferred.

Results

Although there were minimal differences in fermentation profiles and nutritional aspects between treatments across the sampling days, the application of Tx was evident with increases in 1,2-propanediol content at days 0 and 12 relative to the feeding trial ($P \geq 0.05$). Furthermore, ethanol content was slightly affected by Tx application in samples collected on day 12 ($P = 0.014$). Despite the minimal differences in fermentation profiles, yeasts were significantly lower in Tx corn silage collected at 50 cm on day 0 and for both sampling heights on day 12 ($P = 0.009$, $P = 0.005$, and $P = 0.05$ respectively). Furthermore, LAB counts were higher in Tx corn silage at 150 cm sampling depth on day 12 ($P = 0.05$). Subsequently, aerobic stability was affected by treatment in samples collected on day 0 at the 50 cm sampling depth. Application of Tx

reduced the time spent above the ambient temperature threshold of 2°C as well as the deterioration coefficient ($P = 0.03$ and $P = 0.009$, respectively). However, no Tx differences were detected across samples on the day 6 sampling ($P = 0.33$ and $P = 0.25$; 50 and 150 cm respectively). During the 12-day experimental feeding period, Con silage after 24 hours of aerobic exposure (C24) was less preferred by the cows compared to fresh silage regardless of treatment (C0 and T0). Additionally, cows preferred the Tx silage after 24 hours of aerobic exposure (T24) compared to C24 when analyzing with a 60% and 70% threshold.

Table 2: Estimated palatabilities relative to C0

Threshold	Comparisons	C0	C24	T0	T24
50%	191	0.00 ^{ab}	-0.72 ^c	0.25 ^a	-0.31 ^{bc}
60%	168	0.00 ^{ab}	-0.81 ^c	0.28 ^a	-0.26 ^b
70%	141	0.00 ^a	-0.82 ^b	0.10 ^a	-0.19 ^a
80%	112	0.00 ^a	-0.64 ^b	0.03 ^a	-0.16 ^{ab}

^{abc}Superscripts indicate statistical significance ($P \leq 0.05$).

Conclusions

Although inoculant use seemed to have minimal impact on the fermentation and nutritional composition of corn silage in this experiment, there were some subtle signs of Tx application. This includes higher concentrations of an intermediate metabolite that can be indicative of heterofermentation. Despite these minimal differences, yeast counts were reduced with subsequent improvements in aerobic stability. Additionally, Tx seems to have influenced silage palatability as measured through differences in dry matter intake. Although further work is needed to fully characterize additional organoleptic aspects in silage that could influence feeding preference in animals, this research demonstrates the application of inoculants can provide benefits beyond the bunker.

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Unraveling Silage Fermentation with Metagenomics and Metabolomics

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Keywords: OMICs, Silage microbiology, Metagenomics, Metabolomics, community analysis, functional analysis

Introduction

Although the practice of ensiling dates back thousands of years, it remains effective today to preserve large quantities of forages for livestock feeding. Great efforts have since been made to understand silage fermentation. Although publications mentioning silage date back to 1901, some of the first mentions of microbiology in silage did not occur until the 1950's. Concurrently, the first attempt at sequencing was performed in 1953 on two chains of insulin protein, and DNA sequencing was achieved in 1976 with *Saccharomyces cerevisiae* (Giani et al., 2020). Sequencing approaches did not seem to be applied for silage research until the 1970's.

From those first attempts, improvements in analytical approaches to reduce sequencing errors and allow for high-throughput analysis have not only increased the availability but also reduced the cost of sequencing approaches (Giani et al., 2020). Further improvements in sequencing, including next generation sequencing (NGS) and omics-based approaches, are going to reduce the complexity and cost which will increase access for researchers.

According to PubMed, there are now close to 400 silage-related publications with the mention of 'microbiome'. Although this number is increasing for silage, similar efforts to study the rumen microbiome occurred much earlier. Considering the importance of silage in ruminant diets, which can comprise between 50 and 70% of a total mixed ration for dairy cattle, it is necessary to similarly characterize silage microbiology with the high degree of precision offered by omics-based approaches. These techniques will not only continue to help advance knowledge on ensiling, but will also contribute to developing new tools and technologies to address challenges.

Defining OMICS and OMICS-based approaches

Simply put, the suffix 'ome' and 'omics' in a biological context refers to the study of a group of molecules. Furthermore, omics also refers to "analyzing large amount of data representing structure and function of an entire makeup of a given biological system at a particular level" (Dai and Shen, 2022). For instance, genomics is the study of a given genome whereas transcriptomics studies total RNA. Furthermore, proteomics is the study of proteins expressed and metabolomics is the sum of total metabolites in a given system. From these disciplines, there are several other 'sub-disciplines' including glycomics and volatomics. Collectively, these disciplines can contribute to a greater understanding of a given system in the context of 'who' is active and 'what' is happening. Finally, adding the prefix 'meta' expands the analysis from a single specie to multiple in a given system. While the application of omics is not new in agriculture, it seems to have been applied to silage research within the last 10 years.

Understanding individual microbes with genomics, and community structure through metagenomics

Analysis of nucleic acids from silage is not new and has been implemented in silage research to characterize individual strains (genomics) and multiple microorganisms (metagenomics). The implementation of next generation sequencing (NGS) has increased the sample throughput and accuracy, which has helped improve access to researchers. The application of genomics is beneficial for characterizing individual strains of bacteria and fungi, and is an effective tool that can be used to screen new strains as well as thoroughly characterize existing strains. Although metagenomics lacks the sequencing depth offered by whole-genome based approaches in genomics, metagenomics can rely on specific marker genes in complex samples to identify several species. This application is useful in silage research to characterize community diversity and composition across different ensiling conditions.

Functionality through characterizing the transcriptome, proteome, and metabolome

While metagenomics is an effective tool to characterize the microbial community composition, these approaches cannot fully answer functionality of microbial species. Even with the use of whole genome/ shotgun sequencing approaches, it is difficult to describe and report functionality without knowing which genes and when they will be expressed. However, there are other sequencing and identification approaches that provide detailed insight into functional profiles of specific microorganisms in ensiling studies. For instance, the application of transcriptomics/ metatranscriptomics in silage has helped characterize the gene expression of various microorganisms during ensiling. Furthermore, data on protein composition (proteomics) and metabolites (metabolomics) identified in silage samples can help describe the functional activity occurring in silage. Data on the function(s) of specific microorganisms from metatranscriptomics, metabolomics, metaproteomics, and other subdisciplines can complement results from metagenomic approaches for a complete view on ensiling.

Technical considerations and Limitations

Indeed, the application of OMICS and "meta-OMICS" has contributed to improved precision in silage microbiology research, these methods are not free of limitations. For instance with metagenomic approaches, the method of extraction and library preparation will influence the final results (McAllister et al., 2018). This same principal applies for sample

preparation across all omics-based disciplines. Furthermore, there are several steps during the bioinformatics stage that can influence the output of data as well as the interpretation.

Future perspectives

Although silage microbiology publications were published as early as the 1950's, the application of 'OMICS' and "meta-OMICS" was not applied in silage until after 2010. Considering these applications in silage microbiology is more recent compared to other disciplines in animal agriculture, further work is needed. Especially as the technology continues to improve, researchers will benefit from reduced barriers to access these technologies for ensiling trials. While there are some limitations to consider, the overall benefits from omics-based approaches include continuing contribution in understanding silage microbiology as well as helping develop specific solutions and technologies to address challenges.

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Effect of a Combination Homolactic and Heterolactic Inoculant on Alfalfa Fermentation

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Keywords: alfalfa silage, lactic acid bacteria, organic acids.

Introduction

Inoculants with homolactic acid-based microorganisms can increase the speed and improve the efficiency of silage fermentation. Simultaneous inclusion of heterolactic acid-based microorganisms can improve the aerobic stability at feedout. Our objective was to evaluate the effects of a microbial inoculant containing a mixture of *Lentilactobacillus buchneri*, *Lactiplantibacillus plantarum* and *Lactococcus lactis* on the fermentation profiles of alfalfa in laboratory bag silos.

Materials and Methods

Alfalfa was harvested at late bud stage, wilted to 39% DM and chopped. Alfalfa was split into 10 replicate piles (5 per treatment) that were treated with 1 of 2 additives, either water (CTRL) or 300,000 cfu/g of a mixture of *Lentilactobacillus buchneri*, *Lactiplantibacillus plantarum* and *Lactococcus lactis* (BA, Bonsilage Alfa, Provita Supplements). Approximately 800 g of forage from each pile was placed in multiple 25.4 × 35.6 cm nylon/polyethylene bags and vacuum sealed. Bags were stored at 30°C and opened following 1, 2, 5, 7, 56, and 112 d of ensiling. At the time of opening samples were analyzed for nutrient content, and water extracts were collected to measure pH and concentrations of lactic acid bacteria (LAB), enterobacteria, yeasts (d 5-112 only), silage acids, ammonia-N (NH₃-N), and water-soluble carbohydrates (WSC; d 56 and 112 only). Data were analyzed in JMP as a completely randomized design with main effects of 2 inoculant treatments (INO: CTRL vs. BA), 6 days of ensiling (D: 1, 2, 5, 7, 56, and 112), and their interaction.

Results

Dry matter, pH, LAB, yeasts, enterobacteria, and NH₃-N were all affected by the main effects of INO, D, and their interaction ($P < 0.05$; Table 1). Water soluble carbohydrates were affected by treatment and the interaction of treatment by day ($P < 0.05$). On all days, DM was greater for BA than CON, but the DM of BA did not change over time while DM of CON decreased over time with ensiling. pH was lower for BA than CON at all time points with the most dramatic difference of 1.7 units at D2. Concentration of LAB was greater for BA than CON on D1 and D2, but no differences were observed thereafter. Yeasts were greater for CON than BA on D5 and D7, and enterobacteria were greater for CON than BA on D2, D5, and D7. Ammonia-N was greater for CON than BA on D2 through D112, and WSC were greater for BA than CON on D56 and D112.

Organic acids, the ratio of lactic acid to acetic acid (LA/AA), 1,2-propanediol, and ethanol were all affected by the main effects of INO, D, and their interaction ($P < 0.05$; Table 1). Lactic acid increased over time much more dramatically for BA than CON, and was greater for BA at D1, D2, D5, and D7. Acetic acid increased for both treatments over time but more so for CON, resulting in greater acetic acid for CON than BA at D5 and D112. The ratio of LA/AA was greater for BA than CON D1 through D7 but did not differ on D56 or D112. Propionic acid was greater for CON than BA on D2 through D112. 1,2-propanediol was greater for BA than CON on D56 and D112. Ethanol was greater for CON than BA on D5 through D112.

Table 1. Effect of treatment (CON or BA) on dry matter (%), pH, lactic acid bacteria (LAB, log₁₀cfu/g), yeasts (log₁₀cfu/g), enterobacteria (log₁₀cfu/g), ammonia-N (NH₃-N, % DM), and water-soluble carbohydrates (WSC, % DM) of alfalfa ensiled for 1, 2, 5, 7, 56, and 112 d.

	DM	pH	LAB	Yeasts	Entero-	NH ₃ -N	WSC
Day - Treatment							
D1 - CON	39.15 ^{bc}	6.38 ^a	7.81 ^d	--	7.70 ^a	0.03 ^f	--
D1 - BA	40.15 ^a	5.77 ^b	9.17 ^b	--	7.44 ^a	0.03 ^f	--
D2 - CON	38.77 ^{cd}	6.34 ^a	8.68 ^c	--	7.99 ^a	0.06 ^e	--
D2 - BA	40.06 ^{ab}	4.65 ^d	9.61 ^a	--	6.72 ^b	0.04 ^f	--
D5 - CON	38.51 ^{cd}	4.80 ^c	9.33 ^{ab}	3.60 ^a	3.02 ^c	0.13 ^c	--
D5 - BA	40.62 ^a	4.34 ^f	9.50 ^{ab}	2.67 ^{bc}	< 2.00 ^d	0.06 ^e	--
D7 - CON	37.88 ^{de}	4.73 ^{cd}	9.21 ^b	3.14 ^{ab}	2.75 ^c	0.14 ^{bc}	--
D7 - BA	40.18 ^a	4.30 ^f	9.38 ^{ab}	2.27 ^{cd}	< 2.00 ^d	0.07 ^e	--
D56 - CON	37.90 ^{de}	4.50 ^e	8.09 ^d	2.04 ^{cd}	< 2.00 ^d	0.16 ^a	1.55 ^b
D56 - BA	39.84 ^{ab}	4.33 ^f	7.99 ^d	2.01 ^{cd}	< 2.00 ^d	0.09 ^d	2.32 ^a
D112 - CON	37.48 ^e	4.50 ^e	7.17 ^e	< 2.00 ^{de}	< 2.00 ^d	0.14 ^b	1.70 ^b
D112 - BA	40.05 ^{ab}	4.32 ^f	7.12 ^e	< 2.00 ^e	< 2.00 ^d	0.10 ^d	2.17 ^a
SEM	0.19	0.02	0.08	0.16	0.13	0.003	0.06
P values							
INO	0.001	0.001	0.001	0.001	0.001	0.001	0.001
D	0.001	0.001	0.001	0.001	0.001	0.001	0.98
INO × D	0.001	0.001	0.001	0.04	0.001	0.001	0.03

^{a-f}Within column, values with unlike superscripts differ ($P \leq 0.05$).

Table 2. Effect of treatment (CON or BA) on concentrations of organic acids, 1,2-propanediol, and ethanol and the ratio of lactic acid to acetic acid of alfalfa ensiled for 1, 2, 5, 7, 56, and 112 d.

	Lactic	Acetic	LA/AA	Propionic	Butyric Acid	1,2-	Ethanol
Day - Treatment							
D1 - CON	0.13 ^h	0.42 ^e	0.30 ^g	0.04 ^c	0 ^c	0 ^e	0.27 ^f
D1 - BA	1.71 ^g	0.33 ^e	5.18 ^c	0.03 ^c	0 ^c	0 ^e	0.28 ^f
D2 - CON	0.83 ^{hg}	0.73 ^{de}	1.15 ^{fg}	0.26 ^a	0 ^c	0 ^e	0.39 ^{de}
D2 - BA	4.99 ^f	0.49 ^{de}	10.3 ^a	0.10 ^{bc}	0 ^c	0 ^e	0.33 ^{ef}
D5 - CON	5.79 ^{ef}	1.65 ^{bc}	3.50 ^{de}	0.19 ^{ab}	0 ^c	0.003 ^e	0.80 ^{ab}
D5 - BA	8.32 ^b	0.96 ^{de}	8.66 ^b	0.04 ^c	0.01 ^{bc}	0.02 ^e	0.44 ^{cd}
D7 - CON	6.63 ^{de}	1.76 ^{bc}	3.77 ^{cde}	0.27 ^a	0.002 ^{bc}	0.01 ^e	0.85 ^a
D7 - BA	9.68 ^a	1.13 ^{cd}	8.59 ^b	0.06 ^c	0.08 ^a	0.03 ^{de}	0.49 ^c
D56 - CON	7.54 ^{bcd}	2.24 ^b	3.37 ^{de}	0.26 ^a	0.02 ^{bc}	0.09 ^d	0.74 ^b
D56 - BA	7.64 ^{bc}	1.67 ^{bc}	4.57 ^{cd}	0.04 ^c	0.02 ^b	0.35 ^b	0.43 ^{cd}
D112 - CON	7.49 ^{bcd}	3.19 ^a	2.48 ^{ef}	0.22 ^{ab}	0.01 ^{bc}	0.21 ^c	0.78 ^{ab}
D112 - BA	6.88 ^{cd}	2.23 ^b	3.34 ^{de}	0.06 ^c	0.01 ^{bc}	0.50 ^a	0.46 ^c
SEM	0.19	0.14	0.30	0.03	0.005	0.01	0.01
P values							
INO	0.001	0.001	0.001	0.001	0.001	0.001	0.001
D	0.001	0.001	0.001	0.001	0.001	0.001	0.001
INO × D	0.001	0.04	0.001	0.003	0.001	0.001	0.001

^{a-f}Within column, values with unlike superscripts differ ($P \leq 0.05$).

Conclusions

Despite being a combination inoculant, the BA treatment resulted in a very strong lactic acid response, causing a more rapid rise in LAB and lactic acid and a more rapid decline in pH than the CON treatment. Although the BA treatment had numerically or significantly lower acetic acid than the CON treatment at all time points, the higher 1,2 propanediol found for the BA treatment at the later times demonstrates activity of *Lentilactobacillus buchneri* from the inoculant. A more favorable fermentation was driven by the BA treatment as evidenced by lower pH, yeasts, enterobacteria, NH₃-N, and ethanol, and greater WSC and lactic acid compared to CON.

Effect of a Combination Homolactic and Heterolactic Inoculant on Bacterial Community of Alfalfa Silage

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Keywords: alfalfa silage, lactic acid bacteria, microbiome.

Introduction

Lactic acid bacteria (LAB) are known to be efficient silage inoculants. Addition of homolactic LAB to forages with high buffering capacity such as alfalfa can speed the fermentation process to disallow growth of unfavorable organisms and preserve forage nutrients. Combination products also containing heterolactic LAB produce acetic acid that inhibits yeast and mold growth, increasing stability during feed out. The objective of this experiment was to evaluate the effect of a combination inoculant containing *Lentilactobacillus buchneri*, *Lactiplantibacillus plantarum* and *Lactococcus lactis* on changes in the bacterial profile of alfalfa during ensiling.

Materials and Methods

Alfalfa was harvested at late bud stage, wilted to 39% DM and chopped. Alfalfa was split into 10 replicate piles (5 per treatment) that were treated with 1 of 2 additives, either water (CTRL) or 300,000 cfu/g of a mixture of *Lentilactobacillus buchneri*, *Lactiplantibacillus plantarum* and *Lactococcus lactis* (BA, Bonsilage Alfa, Provita Supplements). Approximately 800 g of forage from each pile was placed in multiple 25.4 × 35.6 cm nylon/polyethylene bags and vacuum sealed. Bags were stored at 30°C for 2, 5, and 112 d (D2, D5, D112).

Microbial DNA was extracted from D0 fresh forage and D2, D5 and D112 alfalfa silage cell pellets using the QIAamp PowerFecal Pro DNA Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Frozen DNA samples were sent to RTLGenomics (Lubbock, TX, USA) for sequencing of the V4-V5 region of 16S rRNA genes using universal primers 515yF/926pF using Illumina MiSeq. Sample sequences were denoised and filtered using DADA2 in QIIME2, and taxonomic assignments were made against the SILVA_138.2_99_16S database. Amplicon sequence variant (ASV) tables were exported to R and normalized to the median sequencing depth (8538 bp). Alpha diversity measures (Observed species and Shannon index) were calculated using the normalized ASV table and the "estimate richness" function in the 'phyloseq' RStudio package. Differences were tested with ANOVA and Tukey's multiple pairwise tests Bray-Curtis dissimilarity and weighted UniFrac principal coordinate analysis (PcoA) plots were generated with normalized ASV tables. Statistical analysis of the distance matrices was completed using the 'adonis' function in the 'vegan' R package. Analysis of distance matrices were determined using the 'betadisper' function and differences among treatments and days was determined using PERMANOVA. Taxa bar plots were created using the 'microbiome' and 'microbiomeutilities' R packages and averaging by treatment. Treatment differences in bacterial abundance were statistically analyzed using Wilcoxon rank sum tests.

Results

For both alpha diversity matrices, the D0 sample had the greatest diversity, CON silage intermediate, and BA the lowest (Figure 1). There were no differences over time observed for BA, but alpha diversity was lower at D112 than D5 for CON ($P < 0.01$ for both indices).

Beta diversity matrices revealed significant differences among all treatments and timepoints across both Bray-Curtis and weighted UniFrac measures ($P < 0.01$), and the weighted UniFrac PCoA plot is shown in Figure 2.

Relative abundance of bacterial genera was highly impacted by treatment (Table 1). By day 2, CON silage was predominantly comprised of *Weisella* whereas BA contained almost 95% *Lactiplantibacillus*. This demonstrates that the inoculant in the BA treatment rapidly dominated the fermentation process. *Lentilactobacillus* increased slowly for the BA treatment, reaching 1.7% at D5 and 18.4% at D112. This is in contrast to the CON treatment that reached only 5.4% on D112.

Figure 1. Effect of treatment and day on alpha diversity measures.

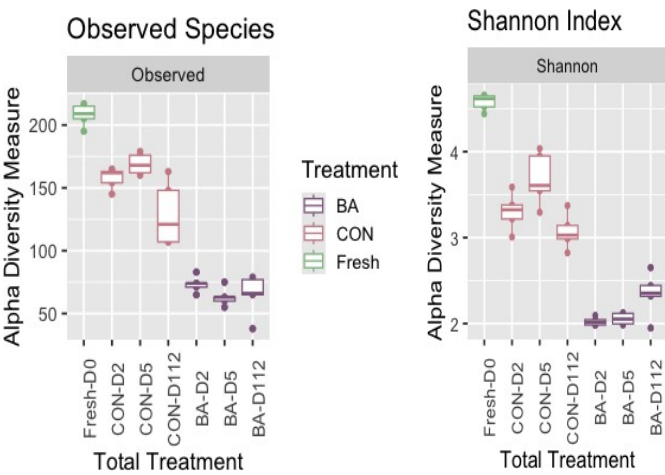


Figure 2. Effect of treatment and day on weighted UniFrac measure of beta diversity.

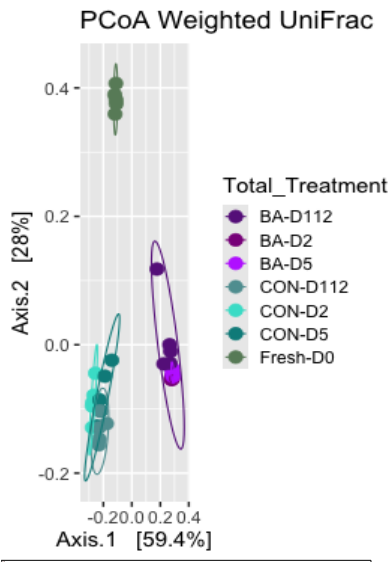


Table 1. Effect of treatment and day on relative abundances (% of total)

Genus	Treatment-Day					
	BA-D2	BA-D5	BA-D112	CON-D2	CON-D5	CON-D112
<i>Enterococcus</i>	0.1 ^c	0.1 ^c	0.2 ^c	4.2 ^a	1.8 ^b	2.4 ^{ab}
<i>Lactiplantibacillus</i>	94.5 ^a	93.9 ^a	70.8 ^b	0.4 ^e	8.0 ^c	4.0 ^d
<i>Lactobacillus</i>	0.0 ^b	0.1 ^b	0.2 ^b	0.2 ^b	7.7 ^a	1.8 ^{ab}
<i>Lactococcus</i>	2.5 ^a	0.6 ^b	0.2 ^c	3.6 ^a	1.0 ^b	0.3 ^c
<i>Latilactobacillus</i>	0.9	0.4	0.4	0.5	1.2	0.6
<i>Lentilactobacillus</i>	0.0 ^d	1.7 ^c	18.4 ^a	0.0 ^d	0.2 ^d	5.4 ^b
<i>Leuconostoc</i>	0.0 ^c	0.0 ^c	0.0 ^c	0.4 ^a	0.1 ^{bc}	0.2 ^b
<i>Levilactobacillus</i>	0.0 ^{cd}	0.0 ^d	0.0 ^d	0.1 ^c	1.4 ^a	0.6 ^b
<i>Loigolactobacillus</i>	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.5 ^b	0.3 ^b
<i>Pediococcus</i>	0.0 ^d	0.0 ^d	0.0 ^d	0.1 ^c	0.9 ^a	0.4 ^b
<i>Weissella</i>	0.8 ^b	0.8 ^b	3.5 ^b	72.3 ^a	68.5 ^a	79.9 ^a

of genera.

Conclusions

Addition of the BA inoculant resulted in a very different bacterial profile compared to the CON inoculant. The inoculant organisms drove fermentation, resulting in lower alpha diversity for BA compared to CON and a greater abundance of the genus *Lactiplantibacillus* and *Lentilactobacillus*. The CON silage was dominated by *Weissella* and had greater abundance of *Enterococcus*, *Levilactobacillus* and *Pediococcus* compared to BA.

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Ensuring Quality Feed Through Silage Making for Smallholders

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Keywords: Silage, Animal Feed, Smallholders, Sustainable Agriculture, Livestock Nutrition

Introduction

The increasing frequency of climatic and economic uncertainties necessitates innovative approaches to livestock feeding (Bateki *et al.*, 2020). Silage-making offers smallholder farmers a reliable method for sustaining livestock feed (Sahoo (2018). While it is beneficial during lean periods by preserving green fodder through fermentation (Balehegn *et al.*, 2022). This study explores key silage-making techniques and their benefits for smallholder farmers.

Materials and methods

This study reviews silage-making practices, focusing on ingredient selection, silo construction, forage preparation, and storage techniques. Emphasis was placed on combining crops such as maize, sorghum, and legumes to enhance feed quality. Key parameters like moisture content and compaction methods were analyzed.

Results

The analysis highlights the benefits of silage, including nutrient retention, digestibility, and reduced storage requirements compared to hay. Table 1 summarizes key metrics, such as optimal moisture content (60-70%) and economic considerations like preparation time (~10 hours for 2,000 kg silage). Economic losses (~15-20%) were identified as a critical factor requiring improved preparation and storage techniques.

Table 1: Summary of Key Silage-Making Metrics for Smallholders

Parameter	Value/Details	Notes
Recommended Crops	Maize, Sorghum, Alfalfa, Grass-Legume Mixtures	Maize yields ~10 tons of silage per hectare; sorghum suited for dry conditions.
Optimal Moisture Content	60-70%	Ensures proper fermentation; too high leads to butyric acid formation.
Silage Density	~400 kg/m³	Affects silo space requirements; compaction is key to quality preservation.
Silo Types	Stack, Trench, Bunker, Plastic Bags	Selection based on resources and scale of operation.
Preparation Time	~10 hours for 2,000 kg silage with 2 workers	Includes harvesting, transporting, chopping, compacting, and sealing.
Additives	Molasses (9 kg/500 kg silage), Bacterial Inoculants	Enhances fermentation and increases palatability.
Storage Duration	Minimum 60-70 days fermentation	Longer storage improves digestibility and reduces spoilage risks.
Economic Losses	~15-20% during ensiling process	Losses can increase with poor preparation or improper storage.
Benefits	Higher livestock productivity, reduced seasonal feeding gaps, better fertility outcomes	Includes increased milk/meat yield and healthier livestock.

Conclusion

Silage-making represents a transformative solution for smallholder farmers, ensuring consistent livestock productivity amidst environmental and market disruptions. By adopting recommended practices, farmers can achieve higher livestock productivity and economic stability.

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Metatranscriptomic Insights into Enhanced Silage Fermentation and Safety by *Lactiplantibacillus plantarum*

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Keywords: Transcriptomic, gene expression, corn silage

Introduction

Silage fermentation is a critical process in agricultural production, that is influencing feed quality and safety. The microbial composition and functionality during fermentation determine nutrient availability and potential pathogen risks. Among silage inoculants, *Lactiplantibacillus plantarum* (LP) has garnered attention for its ability to enhance fermentation quality (Xu et al. 2022). However, its influence on the transcriptomic landscape of microbial communities remains poorly understood. This study explores the temporal transcriptomic impact of *Lactiplantibacillus plantarum* (LP) on microbial community dynamics and functional gene expression, in whole-crop corn silage at 3 days (3D) and 90 days (90D) of ensiling.

Materials and methods

Whole crop corn silage (*Zea mays* L.) was harvested in Dingxi City, Gansu Province, China. Freshly chopped corn samples were randomly divided into four subsamples as treatments and fermentation duration (3 and 90 days), with three replicates for each treatment. One treatment served as control with no inoculants, while one set received LP (*Lactiplantibacillus plantarum*) as an inoculant. Approximately 300 g of fresh corn per bag was ensiled in vacuum-sealed polyethylene bags (30x23 cm) for each sample. Metatranscriptomic analysis was performed by extracting total RNA and removing ribosomal RNA. High-quality RNA (RIN ≥ 5.5) was used to construct macro-transcriptome libraries with the TruSeq Stranded mRNA LT Sample Prep Kit, followed by sequencing on the Illumina NovaSeq platform (PE150).

Results

Microbial community analyses showed distinct shifts in relative microbial abundance across treatments. LP-treated silage in 3D was dominated by *Lactiplantibacillus plantarum* (>75% of the community), which established itself early and at 90D was replaced by *Lactobacillus acetotolerans*. In contrast, control samples on the 3rd day were also dominated by *Lactiplantibacillus plantarum* but at 90D replaced by *Lentilactobacillus buchneri*. LP-treated silage also displayed reduced microbial diversity but enhanced stability, aligning with its superior fermentation quality.

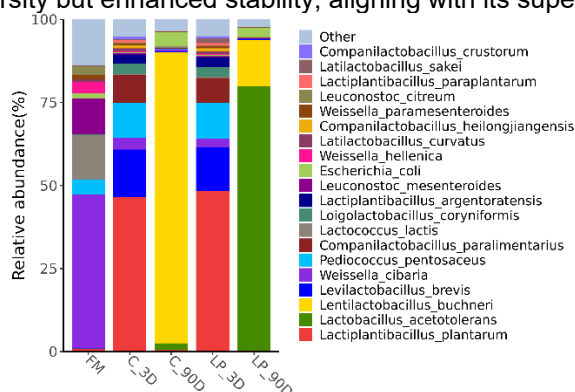


Fig 1 Microbial community abundance bar plot of top 20 species

Transcriptomic profiling revealed significant differences in functional gene expression between LP-treated and control samples at both 3D and 90D of ensiling. At 3D, LP-treated silage (LP_3D) showed elevated expression of glycosyl hydrolases such as GH70 and GT106, indicating an early stimulation of polysaccharide degradation. Control samples (C_3D) exhibited slower metabolic activation, with fewer carbohydrate-active enzymes expressed. By 90D, LP-treated silage (LP_90D) displayed a broader range of highly expressed CAZY enzymes, including GH152 and GH78, highlighting LP's ability to sustain and amplify carbohydrate metabolism over time.

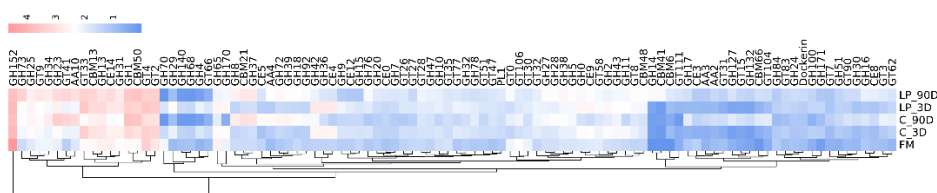


Fig 2 Heatmap of CAZy enzymes among treatments

Virulence factor analysis revealed that LP treatment reduced the expression of key virulence-associated genes, including *fimA*, *ompA*, and *flgA*, particularly at 90D. In contrast, control samples (C_90D) retained higher levels of these virulence factors, suggesting a persistently higher risk of microbial pathogenicity. These findings demonstrate LP's progressive role in improving microbial safety during extended ensiling.

Metabolic pathway analysis using KEGG revealed that LP treatment induced a time-dependent modulation of silage microbiota. At 3D, LP treatment enhanced the expression of genes related to carbohydrate metabolism and early fermentation processes. By 90D, LP further amplified these effects, while simultaneously suppressing genes associated with antibiotic resistance pathways and virulence. Control samples showed a delayed metabolic response, with fewer beneficial metabolic pathways activated at both time points.

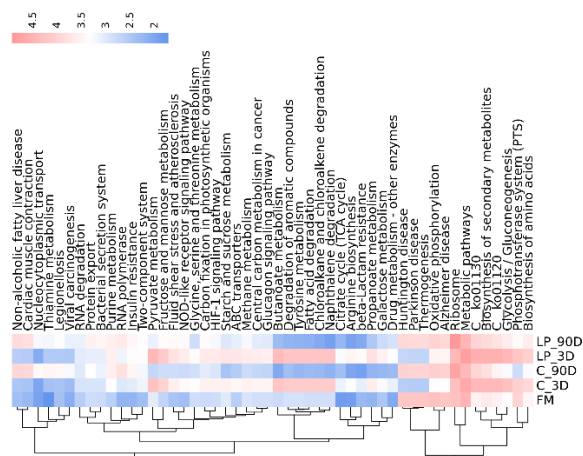


Fig 3 Heatmap of metabolic pathway among treatments

Conclusions

The study highlighted the critical role of LP in modulating microbial community and metabolic activity during silage fermentation. The results underscore LP's dual role in accelerating early-stage fermentation and stabilizing the microbial community during prolonged ensiling, resulting in improved silage quality and reduced the risks of microbial pathogenicity.

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Utilizing Indigenous LAB Inoculants for Ensiling Rice Straw with Agro-industrial Byproducts and Evaluation of Its Characteristics

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Keywords: Stubble burning, crop residues, aerobic stability, lactic acid bacteria

Introduction

Rice straw, the crop residue left to burn in the fields per se to prepare the fields for the next sowing cycle, is the most abundantly available underutilized feed resource which can be diverted to livestock feeding if efficiently processed. This practice while helping to minimize air pollution, simultaneously will provide an alternate feed resource for livestock. Ensiling is the best-suited intervention to utilize these crop residues as livestock feed but is difficult in the case of rice straw as it is deficient in moisture and soluble carbohydrate content while rich in fibrous carbohydrates, hence is a difficult crop to ensile; with the additional challenge of high temperature causing quicker spoilage in tropical countries. To address these impediments, the current study was undertaken to employ indigenous inoculants with fibrolytic properties for efficient ensiling of rice straw with other agro-industrial byproducts and assess the fermentation characteristics of the obtained TMR silage.

Materials and methods

A combination of paddy straw, potato waste, and wet distillers' grains in a specific ratio with xylanase were used as ingredients and ensiled in mini-silos for making TMR silage. The treatment groups and inoculant combinations employed are shown in Fig.1. Silages were sampled on the 40th, 50th, and 60th day of ensiling for their fermentation characteristics, chemical composition, and microbiology. Aerobic stability of silage was estimated by the change in temperature of the silage measured by recorded readings of data logger through sensor probes after aerobic exposure. Lactic acid content in silage samples was measured as per the method of Barker and Summerson (1941). Short chain fatty acids were estimated using Gas chromatography. Lactic acid bacteria, yeast, mould count and the most probable number of Clostridial spores were counted in fresh silage samples (Tabacco et al., 2009). Aflatoxin B1 content was assessed using Neogen® Raptor® Reveal® Q+max for Aflatoxin B1 kit as per manufacturer protocol. Silage metabolites were evaluated by LC-MS analysis. Bacterial community dynamics was estimated by v3-v4 sequencing. Colonization of silage samples was observed at 5500x using scanning electron microscopy. Principal component analysis was conducted using Metaboanalyst (6.0) to obtain statistical differences and relationship among the treatment groups based on various silage quality and fermentation characteristics.

Results

After 50 days of ensiling, all TMR silages were well formed and had a pH range between 3.60-4.02. The treatment *LcLp* had the highest DM recovery, lowest pH, highest lactic acid content, and the highest aerobic stability, after 50 days of ensiling. The acetic acid and propionic acid content of all additive-treated silages were higher ($p < 0.05$) than the control group, with the highest amount of acetate and propionate present in silage *LcLp*, after 60 days of ensiling. The LAB count was significantly higher in all additive-treated TMR silages than control. The lactic acid bacteria (log 10 CFU/g) count was the highest after 60 days of ensiling, while yeast count was lowest in the group *LcLp* after aerobic exposure. The *Lactobacillus* genera showed the highest relative abundance in the *LcLp* group among all the other treatment groups, while *Lactobacillus fermentum* was the most dominant species discovered in all silages (Fig. 2). 1170 Metabolites were identified using LCMS technique, and 350 of them were found to be significantly expressed in all the treatment groups. Top up-regulated metabolites included DL-Tryptophan, Phenylalanine, Ferulic acid, Isophorone, and Acetylenol, while top downregulated metabolites include Icosanedioic acid, 2-Deoxyhexopyranose, Syringol, p-coumaroylputrescine, mycosporine, α -Chaconine. SEM images (Fig.3) revealed that the highest colonization by rod-shaped bacilli was found in the *LcLp* silage as compared to the other treatment groups. Using principal component analysis, it was revealed PC1 represented 73% of the data and explained the major significant differences, while PC2 represented 19.1% of the data, based on which it was concluded that treatment groups *LcLp*, *LaLf* and RIC strains had better silage quality indices than other groups.

Conclusions

Two combinations of homofermentative and heterofermentative LAB (RSB 5: *Lactiplantibacillus pentosus* and RSB 2: *Lactocaseibacillus casei* with xylanase) and (RSB 7-*Lactiplantibacillus argentoratensis*; RSB 9-*Limisolactobacillus fermentum* with xylanase) proved to be effective inoculants for co-ensiling paddy straw with processed potato waste and wet distillers' grains in the ratio 40:40:20. The best quality TMR silage was obtained after 50 days of ensiling.

Figures:

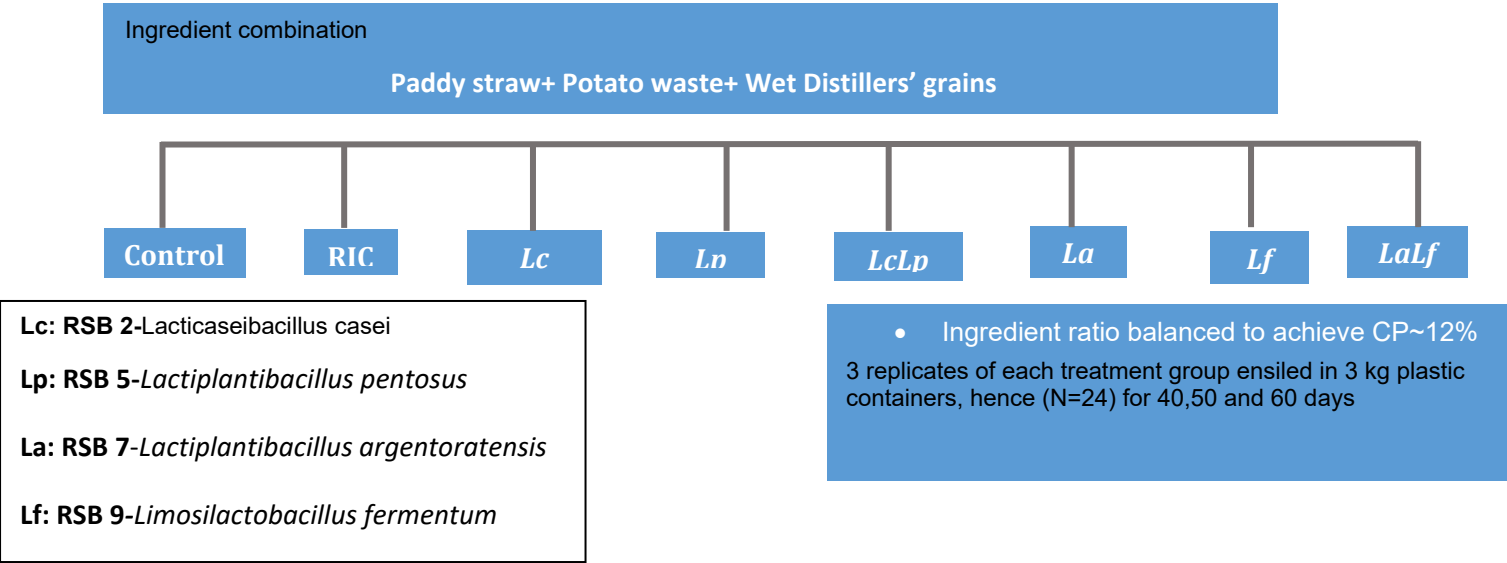


Fig.1 : Preparation of lab scale TMR silage inoculated with the indigenous LAB combinations and its assessment for *in-vitro* fermentation characteristics

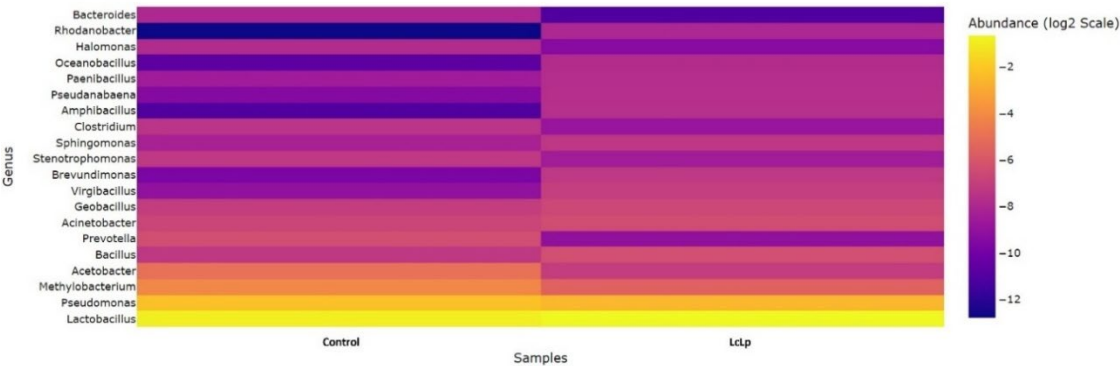


Fig. 2 Dominant genera in TMR-silages inoculates with *LcLp* in comparison to control silage

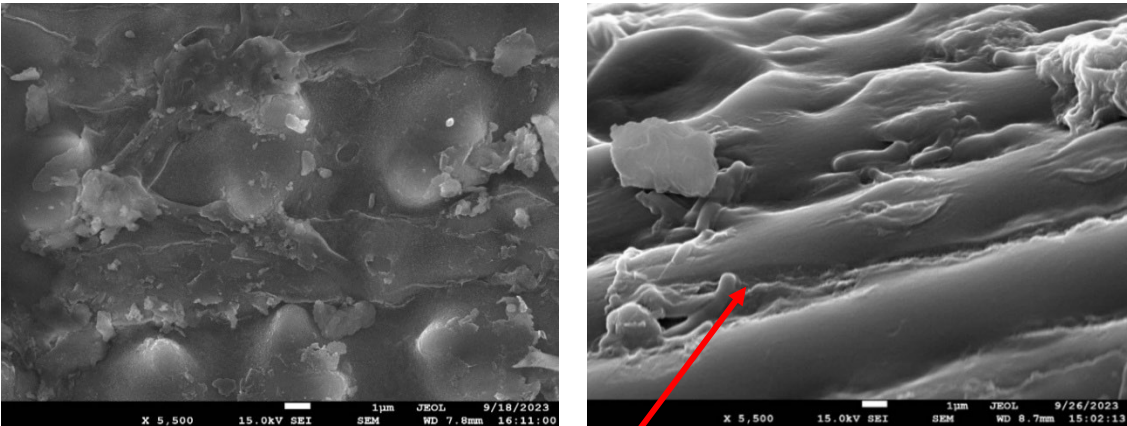


Fig 3 (A)

More colonization by *Lactobacilli*

Fig 3 (B)

Sparse colonization by *Lactobacilli*

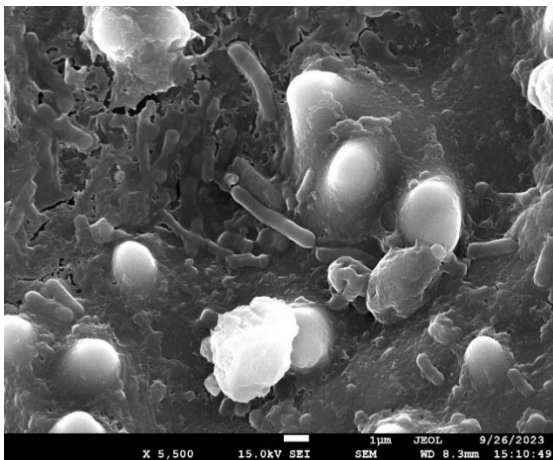


Fig 3 (C)

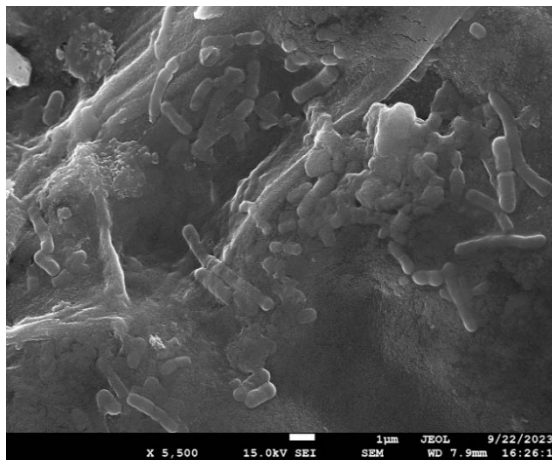


Fig 3(D)

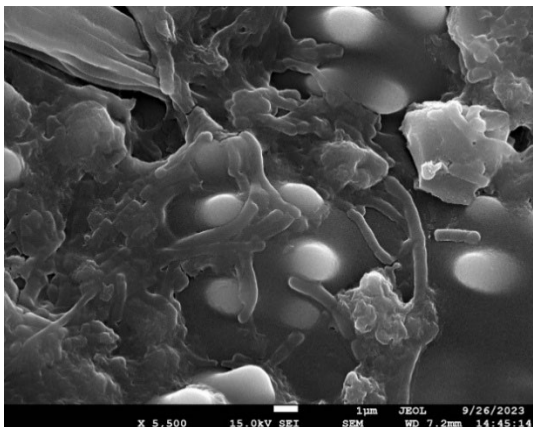


Fig 3 (E)

Fig 3. Scanning electron microscopy of rice straw and native LAB inoculant-treated TMR silages A: Rice straw (non-ensiled); B: Control; C: RIC (Reference inoculant); D: *LcLp*; E: *LaLf*. Magnification: X5500

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Silage Volatile Organic Compounds – Effects on the Environment and Animal Performance

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Keywords: Dry matter intake, silage fermentation, silage metabolites, ruminant

Background

One of the main objectives of preservation of forage is to minimize dry matter (DM), energy, and quality losses to maintain the resource cycle and the nutritional quality of the harvested material. The DM losses during ensiling, storage, and the feed-out period occur simultaneously with shifts in chemical composition of the forage, and are generally accompanied by gaseous emissions, including volatile organic compounds (VOC) and other volatiles formed during ensiling, or effluent losses. Shifts in chemical composition and gaseous emissions can have an – direct or indirect – impact on the environment and animal performance which are briefly delineated below.

Effects on the environment

Silage generates climate- and environmental-relevant gases during anaerobic fermentation and after silo opening. VOC are minor constituents of gaseous emissions from silage, which predominantly consist of CO₂, followed by methane and nitrous oxide formed during the anaerobic phase, whereas VOC such as ethanol and ethyl esters that can be produced at various stages of the whole chain from harvest to the feed-out period, emit after silo opening. The direct effect of CO₂ emission from silage can be regarded as climate-neutral, as it emits from CO₂ that has been captured through photosynthetic pathways recently during forage development and biomass accumulation. However, the indirect carbon footprint of CO₂ emission is proportional to its formation in and release from silage because carbon losses are mass losses such that discounts must be considered per unit of harvested forage, which have to be balanced by additional forage or other dietary ingredients, each having its own carbon footprint. In concert with CO₂, also VOC have an indirect carbon footprint which has received increasing interest only relatively recently. Because VOC can be formed from numerous sources and both biochemical and chemical pathways can be involved, quantification of the indirect environmental effects of VOC formation and emission is a challenging task and results can show a large range depending on which VOC and pathways are concerned. As an example, when VOC such as the major compounds acetic acid and propionic acid are formed from fermentation of monomeric carbohydrates ('sugar'), the CO₂ emission is substantially lower than formation of minor VOC such as esters from ethanol, because ethanol generation is associated with huge CO₂ emissions. Therefore, reduction or prevention of VOC generation and emission is worthwhile, even without considering adverse effects of some VOC on DM intake (DMI) and feed choice behavior. Future studies on climate- and environmental-relevant gaseous emissions from silage may also help to answer the question raised at the 19th International Silage Conference: "Can silage absorb more carbon than it emits during fermentation" (Schmidt and Vigne, 2023)?

Effects on animal performance

During the aerobic, fermentation and feed-out phases of ensiling and silage use, there is a formation of nonvolatile organic acids such as lactic acid, protein degradation compounds, and other microbial metabolites. In addition, a broad spectrum of desirable and undesirable volatile organic compounds (VOC) at different concentrations and with different effects on silage quality are also synthesized. VOC can influence silage preference and DMI via both palatability and physiological effects (osmolality changes, rumen motility, hepatic oxidation, and postingestive feedback). Effects of silage VOC on animal performance are largely indirect, i.e., mediated through the – predominantly negative – impact of VOC on overall DMI, rate of intake, or forage preference in a choice situation. Direct undesirable effects of VOC on animal performance – and health – are much less frequently observed because ruminants possess a degree of nutritional wisdom in the sense that they generally select foods that meet nutritional needs and avoid foods that may cause toxicosis.

A wide variety of VOC are formed during different stages of ensiling, with concentrations typically ranging from 1 to 200 g/kg DM. Despite the inconsistent results, the available evidence from silage intake models suggests that at concentrations found in normal silage, among the VOC, acetic acid and propionic acid (to some extent) might be major contributors to DMI reduction. In contrast, when the VOC are added to the diet at concentrations that are typically present in silage, or when heterofermentative bacteria increased acetic acid in the silage, the impact of acetic acid is even less pronounced. Additionally, apart from silage intake models and infusion studies, unlike acetic acid, propionic acid was rarely used as a pure substance in forage to determine its effect on preference and DMI. Therefore, further studies are needed to determine the influence of propionic acid on preference and DMI. When VOC are tested for their role in DMI, they should be added to a non-fermented diet just before feeding, so that there are negligible losses of VOC to the atmosphere.

Metabolomic analysis has revolutionized the human health and food sector. However, quantifying a detailed silage metabolome is a relatively new approach, and few data are available that linked metabolomic data and silage preference in animals. In addition to routinely measured metabolites, such as lactic acid, acetic acid, propionic acid, and ethanol, several thousand (up to 6000) metabolites, including VOC, can be found in silage. Additionally, the relative abundance of

these volatile metabolites can change depending on some factors, such as ensiled crops or with inoculation of microbes, such as homo- or heterofermentative lactic acid bacteria.

It is remarkable that much emphasis has been placed on the occurrence of VOC in silage but less on the formation or synthesis of VOC, most notably precise knowledge on the formation of non-routinely measured VOC such as aldehydes, esters, and ketones still are very scant. Aldehydes and esters may deserve special attention because they show peculiarities. Measurement of aldehydes is expensive and tedious. Moreover, unlike most other VOC where one can observe a dominant compound within each group, this is not observed in aldehydes. Concerning the generation of esters in silage, there is still some ambiguity on the major route for their synthesis, with some researchers supporting the abiotic formation, while others corroborate the biotic pathway as the major source for their presence. Lactic acid bacteria and yeasts can synthesize esters using different biochemical pathways. Regarding the abiotic pathway, esters such as ethyl lactate are produced at a slow pace, but this compound is rapidly generated when there are surplus amounts of lactic acid and ethanol in the presence of charged hydrogen ions.

Available data suggest that the type of response (i.e., either positive/negative/neutral) to an individual silage VOC is likely similar among different ruminant species. Nevertheless, studies that compare the relative influence of a specific VOC on DMI in two ruminant species simultaneously are lacking. Moreover, it might be difficult to understand the relative magnitude of the impact of a certain VOC on preference or DMI between different ruminant species unless silage containing the same chemical composition (including VOC) is offered simultaneously in an individual trial. Taste perception can vary between ruminant species. For instance, a sweet taste seems to have a positive hedonic value in cattle but not in sheep, and goats are more tolerant of a bitter taste than sheep and cattle. Nevertheless, the change in preferences for different tastes due to previous experiences cannot be completely ignored. As VOC vary in taste, there is a possibility that they can have slightly different palatability effects in different ruminant species. However, whether this has a significant effect on preference or intake differences between different ruminant species and, finally, animal performance, should be studied further.

Conclusions

Future experiments that employ metabolomic analysis, in addition to substantial routine chemical analysis including VOC, may have the potential to reveal other unidentified VOC that can impact silage preference and intake. Discovering these substances and developing proper management techniques to control them may help to reduce undesirable effects on the environment and improve both the productivity and profitability of ruminant production.

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A Multi-sensor and Multi-parameter Bio-information Sampling System for Mini-Silo Ensiling Experiment

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Keywords: Ensiling tests, Multi-sensor, Multi-parameter, Bio-information Sampling System

Introduction

Crop ensiling, dominated by microbial metabolism, refers to a complex biochemical process and it includes initial oxygen depletion, anaerobic fermentation, anaerobic storage and feed-out phases (Kung et al., 2018). To assess the qualities of anaerobic fermentation, the aerobic stability of silage and the efficacy of additives with comparable results under consistent test conditions, German Agricultural Society (DLG) suggests an experimental scheme, including sample volume (1.5-L jar) and the sample replicates ≥ 3 (Pauly and Wyss, 2018). Following this scheme we devised a multi-sensor-based experimental system. To demonstrate its applicability, the multi-parameter results of maize ensiling are presented.

Materials and methods

Figure 1 exhibits the innovated bio-information sampling system for anaerobic-fermentation (a) and aerobic-stability (b) tests. Both tests allow measuring up nine samples (1.5 L glass jar) and this design provides for either: (i) three groups of different samples and each group with three replicates (3×3) or (ii) two different groups, and each group with four replicates (2×4). For each sample, oxygen (O_2), pH and temperature (Tsi) are measured in situ. For feed-out test, a highlight is to hourly measure the emissions of CO_2 and ethanol (EtOH) from the sample array using a robotic e-nose. This sophisticated system thus integrated thirty sensors (ten temperature sensors, nine O_2 electrodes, nine pH electrodes, a CO_2 sensor and an EtOH sensor). To collect multi-parameter data, five loggers were used. Moreover, a mathematical model was developed with converting pH measurements to the contents of lactic and acetic acids (Sun et al., 2024). The ensiling crop was whole maize (dry mass (DM): 387 g kg⁻¹, chopped length: 1 cm, packed DM density: 180 kg m⁻³). The fermentation (Fig. 1a), immediately after harvested, started on Sept. 11, 2024 and the aerobic stability test (Fig. 1b) was conducted from Dec. 6 to 18, 2024, respectively.

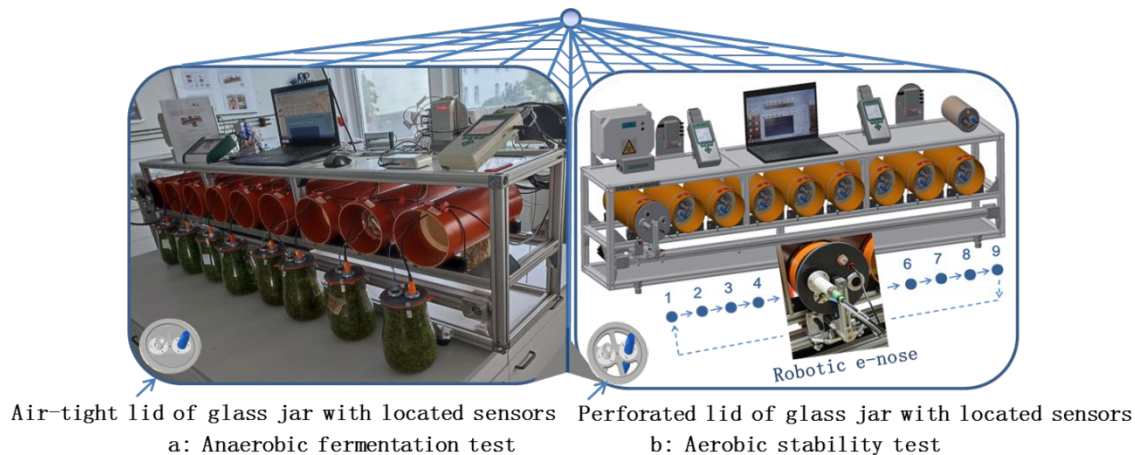


Figure 1: Multi-sensor-based and multi-parameter-combined bio-information sampling system

Results

Multi-parameter data in the anaerobic fermentation (Figure 2) include: initial depletion of O_2 (a), time courses of pH and ΔpH (b), dynamic accumulations of lactic acid (LA) and acetic acid (AA) over time (c), and the relationship of pH and ΔpH (d). Multi-parameter data of the aerobic stability test (Figure 3) include: time courses of O_2 , CO_2 and EtOH (a), those of pH, Tsi and ambient temperature (T_a) (b), differential concentration of O_2 (ΔO_2) and dynamic loss of LA over time (c), and the relationship of O_2 and both CO_2 and Tsi (d). Even though the specific sensors of organic acid (OA) have not been available, we achieved the results of LA and AA (Fig. 2c, Fig. 3c) using a developed pH-OA model (Sun et al., 2024).

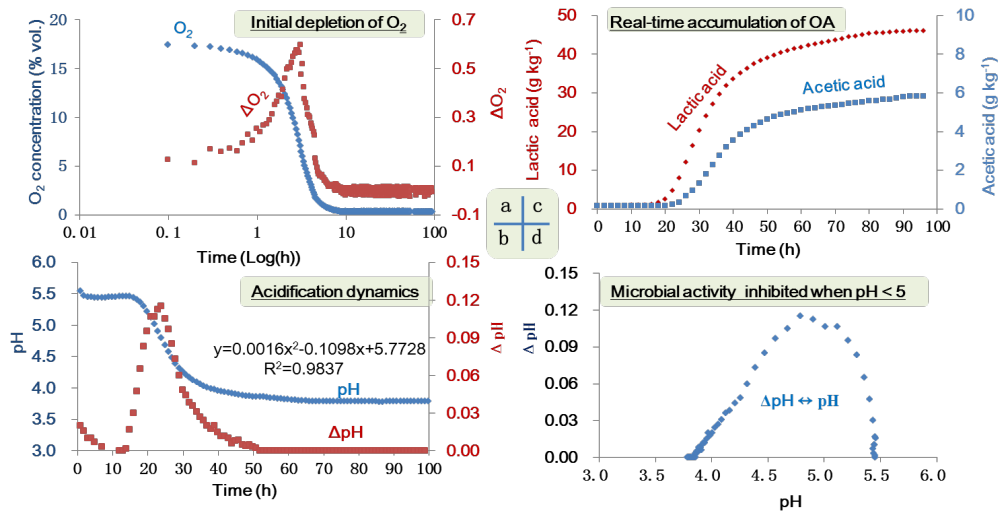


Figure 2: Multi-parameter results of the anaerobic fermentation test

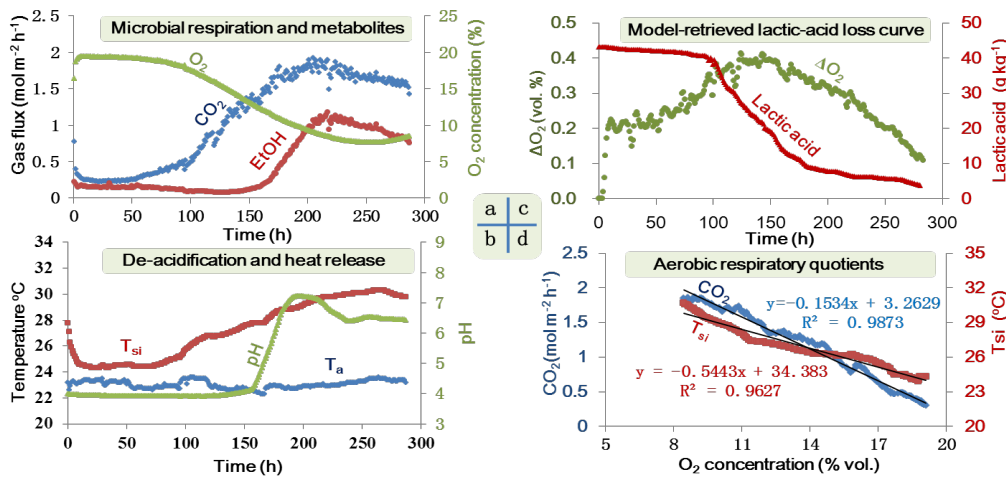


Figure 3: Multi-parameter results of the aerobic stability test

Conclusions

Despite that complete data of nine samples tested are absent due to two-page space, these representative results highlighted technical capacity of the sensor fusion system innovated. The cross interpretation of multi-parameter measurements may provide insight of anaerobic and aerobic microbial metabolisms of crop ensiling, thereby improving our understanding of silage production. The potential use may extend (i) to observe microbial role of additives and (ii) to investigate the effect of gas emission from feed-out silage on local environment.

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Monitoring Changes in Temperature of Fermented Feeds and Their Aerobic Stability

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Keywords: aerobic stability, intake of DM, silage, maize

Introduction

Aerobic stability of silages and Total Mixed Ration (TMR) is a growing concern, particularly during warmer months when feed is more prone to heating and spoilage. Although corn silage is generally stable, mixing it with high dry matter components can accelerate microbial activity and reduce overall stability in practical barn conditions. Most existing studies have been conducted under laboratory conditions, which may not fully reflect real-world feeding systems. The objective of this study was to assess how mixing corn silage with core feeds of higher dry matter content affects the temperature and aerobic stability of TMR under barn conditions.

Materials and methods

At VZOD Zašová (The Czech Republic), we selected two samples of corn silage (sensor 1 and 2) and one sample of total mixed ration (TMR) for dairy cows (sensor 3). The samples were placed in the barn in plastic bags, each containing 5 kg, arranged side by side (see Figure 1). Temperature sensors were placed in the individual samples and sensor 4 for ambient temperature. After inserting the temperature sensors, we turned on the NutriStabil device and connected it to a mobile phone via WiFi. After verifying the device's functionality, we monitored it for a period of 5 days.



Figure 1. The samples in the barn during measuring of the aerobic stability.

Results

Figure 2 below, you can see the recorded temperature changes. During the entire observation period (5 days), the ambient temperature ranged from 19°C to 33°C. In addition to the results of the fermentation process and the nutritional value of the produced corn silages, we measured the temperature at the point of sampling, specifically where the feed is consumed by the animals. The results for both samples of the corn silage (blue and black curves in the graph) indicate an increase in temperature from 22°C (at the start of the measurement) to 43°C within 48 hours. However, the TMR temperature shows an immediate increase after the feed was provided to the dairy cows, as seen in the green curve in the graph. Such a feed ration is already significantly warm to the touch by the evening. As a result of secondary fermentation, i.e. the action of yeast and mold, not only does the feed warm up, but also secondary metabolites are formed, which are poisonous, burden the animal's organism and worsen its health. In addition to the loss of feed value itself, there are also losses arising from the costs of treating the herd and reducing the hygienic safety of preserved feeds.

Considering that the biomass was stored under identical conditions, the difference in fermentation losses is evident. An increase in the temperature of 1 gram of feed by 1°C represents an energy loss of 4.18 J. The original calorie was defined by French chemist Henri Victor Regnault as the amount of heat required to raise the temperature of 1 g of water from 0°C to 1°C. However, in feed analysis, energy values are expressed in Joules or Kilojoules.

The temperature difference between TMR and ambient was 13 °C (in 12 hours). It means that the energy loss of this TMR might be around 3 MJ per dairy cow and day. It depends on the feeding ration.

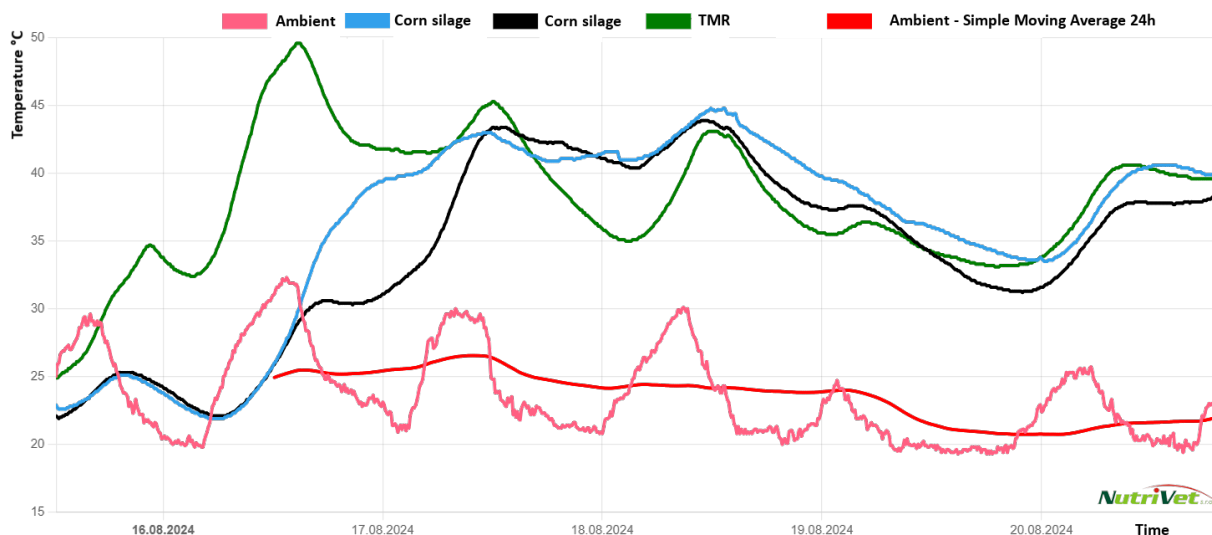


Figure 2: The final chart created by NutriStabil device.

Conclusions

Understanding feed stability under barn conditions is increasingly important due to rising summer temperatures. Our study shows that mixing stable corn silage with high dry matter feeds in TMR significantly increases microbial activity and temperature, reducing aerobic stability—a practical challenge often overlooked in real barn environments. Feed instability can result from high harvest dry matter, long chop length, poor silage covering, and improper feed removal exposing large areas to air. Our findings highlight that preservatives alone do not guarantee stability; proper application and adherence to recommended dosages are essential. The novelty of this work lies in applying lab knowledge to real-world conditions, showing that aerobic stability must be evaluated on-farm. These insights can help improve feeding strategies, staff training, and feed hygiene under heat stress. The results offer practical guidance for future seasons.

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Quality of Silages from Selected Pea and Faba Bean Varieties in the MALÁ HANÁ Region, Czech Republic

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Keywords: silage, nutritional; quality of fermentation; pea; bean

Introduction

Legumes represent an important source of quality protein for animal nutrition (Schumacher et al., 2011). Silages from annual legumes can be a suitable complement to perennial protein crops (Urbano et al., 2007; Turco et al., 2016). The aim of the study was to compare the nutritional values and quality indicators of silages from semi-leafless peas, field pea, and faba bean in the Malá Haná region in 2024.

Materials and Methods

- Six crops were included in the trial:
- Semi-leafless pea Gambit (270 kg/ha)
 - Semi-leafless pea Avatar (270 kg/ha)
 - Leafy-type pea Protecta (270 kg/ha)
 - Field pea Arvika (160 kg/ha)
 - Faba bean Mistral (330 kg/ha)
 - Faba bean Merkur (260 kg/ha)

The trial was conducted in the Velké Pavlovice area at the Velká Routka location (433 m above sea level, soil type brown earth). Rainfall during the vegetation period was low: March 38 mm, April 21 mm, May 73 mm, and June 42 mm. The harvest took place on July 9, 2024.

Results and Discussion

Table 1: Nutritional values of tested legume silages

Parameter	Pea			Bean			SEM
	Gambit	Avatar	Protecta	Arvika	Mistral	Merkur	
DM	17.7 ^b	18.6 ^b	20.3 ^c	25.5 ^d	16.0 ^a	14.8 ^a	0.35
Protein	15.6 ^a	15.4 ^a	15.1 ^a	16.3 ^{ab}	17.9 ^b	15.7 ^a	0.44
NDF	59.5 ^d	58.9 ^d	55.5 ^c	49.5 ^a	50.2 ^{abc}	52.6 ^{bc}	0.67
ADF	45.1 ^c	43.7 ^{bc}	39.7 ^{ab}	34.9 ^a	40.1 ^b	41.8 ^{bc}	1.07
Ash	10.0 ^d	9.7 ^{cd}	9.6 ^c	8.7 ^{ab}	8.8 ^b	8.1 ^a	0.13
Starch	6.1 ^{ab}	5.3 ^a	8.3 ^b	15.1 ^c	6.4 ^{ab}	7.2 ^{ab}	0.52
DOM	65.6 ^a	63.7 ^a	67.7 ^a	78.0 ^b	78.7 ^b	73.2 ^{ab}	1.26

DM = dry matter in % FM. NDF = neutral detergent fiber in % DM. ADF = acid detergent fiber in % DM. Ash and Starch in % DM. DOM = digestibility of organic matter in %. Means within a row (cultivar comparison) not followed by the same letter are different (*P* < 0.05).

Table 2: Fermentation quality indicators of legume silages

Parameter	Pea			Bean			SEM
	Gambit	Avatar	Protecta	Arvica	Mistral	Merkur	
pH	5.21 ^c	5.24 ^c	5.33 ^c	4.54 ^b	3.76 ^a	3.68 ^a	0.07
LA	0.23 ^a	0.12 ^a	0.11 ^a	0.78 ^b	1.30 ^c	1.20 ^c	0.08
VFA	1.44 ^b	1.49 ^b	1.55 ^b	0.85 ^a	0.83 ^a	0.75 ^a	0.07
LA/VFA	0.15 ^a	0.08 ^a	0.07 ^a	0.93 ^b	1.56 ^c	1.61 ^c	0.10
NH ₃ -N	9.76 ^c	8.33 ^c	6.05 ^b	2.44 ^a	2.97 ^a	4.07 ^{ab}	0.50
Losses % DM	15.7 ^{bc}	21.5 ^c	12.6 ^{ab}	7.38 ^a	8.71 ^a	17.3 ^{bc}	1.29

LA = lactic acid in %; VFA = volatile fatty acids in %; NH₃-N in mg N/100 g silage; DM = dry matter, Losses % DM. Means within a row (cultivar comparison) not followed by the same letter are different ($P < 0.05$).

Key findings:

- Among semi-leafless peas. differences were only found in NH₃-N. with the Protecta variety achieving the lowest values.
- Field pea showed better fermentation indicators compared to semi-leafless peas: lower pH. butyric acid content and NH₃-N. higher lactic acid content and LA/VFA ratio.
- Faba bean showed better fermentation indicators than both field pea and semi-leafless peas.
- Compared to the original crop. the silages of tested legumes showed lower dry matter content. NDF and starch. but higher values of crude protein. ADF and organic matter digestibility.

Conclusion

- The results of comparing nutritional values and fermentation quality showed differences both between crops and between their varieties.
- To confirm the results and their practical application. further monitoring will be needed to exclude or confirm the influence of location and weather conditions.
- The results were processed thanks to the financial support of the National Agency for Agricultural Research of the Czech Republic. project no. QK21010344.

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Effect of Theoretical Length of Cut of Sorghum Silage in Backgrounding Beef Steers Performance

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Keywords: beef cattle, sorghum silage, theoretical length of cut

Introduction

Sorghum (*Sorghum bicolor*) crop introduction in beef cattle operations in the southern US has been enhanced by certain agronomical advantages that this species provides to the systems, such as greater drought tolerance, the ability to grow in a wide range of soils, and the potential to produce large amounts of a high-quality feed for cattle (Andewakun et al., 1989). However, utilized as whole-plant silage, Sorghum shows certain limitations when compared with corn (*Zea mays*) silage, like reduced whole-plant digestibility and constraints related to kernel processing. Therefore, to overcome these limitations, it is needed to improve sorghum kernel processing at a farm-scale operation to be fed to backgrounding animals. The factors involved in kernel processing are the type of roll utilized when chopping (related to the number and orientation of teeth), the differential speed between rolls, roll gap and theoretical length of cut (TLC).

An on-farm question that arises when chopping is: What is the proper TLC to be utilized? It is predicted that a shorter TLC would produce a better kernel processing, improving starch digestibility and that would enhance animal performance. That is why in this experiment two different and contrasting TLC were evaluated in the performance of backgrounding beef steers.

Materials and methods

Seventy-two weaned steers (7 ± 2 months of age, 200 ± 40 kg of body weight) were used in a randomized complete block design. Animals were blocked by body weight. The blocking was performed considering that there could be differences in gain and intake due to competition and metabolism between animals related with animal size and weight. That is why animals with similar body weight were assigned to a same pen, and treatments were randomly assigned to pens with similar average body weight and appear in each block only once. The experimental unit was considered the pen. During the experimental period, 72 animals were housed in 12 pens of 100 m² of 6 steers each, with free access to feed and water. Six pens received one treatment diet of Sorghum silage chopped at 7.5 mm of TLC and the other six pens received the other treatment chopped at 15 mm TLC. Both silages were chopped with a Claas Jaguar 870 (Harsewinkel, Germany) harvester, equipped with a conventional Sorghum processor of 19.6-mm diameter rolls, with 125 teeth in both rolls, operating at a differential speed of 50%. The diets were protein corrected with soybean grain mixed with the silage to meet protein requirements (NASEM, 2016). The experimental period consisted of 14 days of adaptation followed by 84 days of intake and body weight measurements and body weight change calculation. Animal performance was assessed by calculating average daily gain (ADG), dry matter intake (DMI) and feed to gain ratio (F:G). Data were analyzed as a randomized complete block design with pen as the experimental unit.

Results

Initial and final body weight did not differ among treatments ($P > 0.05$). Average daily gain and feed to gain ratio were not affected by treatment ($P > 0.05$). However, dry matter intake as a percentage of body weight was increased with the diet containing the sorghum silage chopped with a TLOC of 15 mm ($P < 0.01$). Intake as kg per day tended to be different between treatments ($P = 0.10$), where steers fed the silage chopped at 15 mm tended to show a greater intake of 160 g per day.

Table 1. Effect of sorghum silage theoretical length of cut on average daily gain, intake, and feed to gain ratio in beef steers

Variable	Treatment (TLOC)		SEM ¹	Pr(>F)
	7.5 mm	15 mm		
Initial body weight, kg	205	204	11.1	0.44
Final body weight, kg	253	253	12.1	0.88
Average daily gain, kg	0.575	0.597	0.026	0.49
Intake (% body weight)	3.16	3.24	0.034	0.05
Intake, kg/d	7.26	7.42	0.399	0.10
Feed to gain ratio	12.74	12.44	0.677	0.67

¹. Standard error of the mean.

Table 2. Particle size distribution (%) of each treatment according to the Penn State Particle Separator

Particle size (mm)	Treatment (TLOC)	
	7.5 mm	15 mm
> 19.0	0.6%	6.0%
19.0-8.0	39.5%	51.1%
8.0-1.8	56.7%	40.8%
< 1.8	3.2%	2.1%

Conclusions

This study showed that animals fed Sorghum silage chopped at a shorter length, had similar weight gains with less amount of feed consumed per unit of body weight. This could be implying that shorter length of cut may contribute to a better-quality silage, with better stability and compaction which may be stimulating to reduce refusals or sorting of particles.

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Sorghum Silage as a Feed Alternative for Backgrounding Heifers in Florida

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Keywords: backgrounding, beef cattle, sorghum silage

Introduction

Cattle backgrounding refers to the period of growth between weaning until they enter the feedlot for finishing or enter the herd for the breeding season (replacement heifers). Backgrounding systems in different regions present diverse opportunities and challenges relative to seasonal patterns of cattle production, prices, and forage availability. Although Florida is mostly a cow-calf state, there is a potential opportunity to add value to weaned calves with the use of silage-based diets that allow cattle to be backgrounded and sold at heavier weights at a competitive cost.

Sorghum has been gaining attention in Florida as a crop alternative harvested as whole plant silage for inclusion in backgrounding diets as the main roughage source to fill the forage gap in winter-feeding programs. Sorghum has great advantages over many other summer annual forages. One of them, very important, is its drought tolerance and the plasticity that it has in terms of dry matter production. Being tolerant to drought conditions allows this forage to produce feed even in conditions in which many crops would fail to produce.

Materials and methods

The study was conducted over three consecutive years (2019–2021) at the North Florida Research and Education Center (NFREC), Marianna, Florida. Sorghum (*Sorghum bicolor*) was planted in each year, using different varieties tailored to local conditions and management practices. Crop management, harvesting, and feeding practices varied across years to account for environmental and operational conditions.

Year 1: Forage sorghum hybrid ADV 7401 (Alta Seeds, Irving, TX), was planted on June 9, 2019, targeting 247,000 plants/ha with a 45-cm row spacing. Due to a historical drought, the crop received only 5.28 inches of rainfall, with no supplemental irrigation. Harvest was conducted on October 25, 2019, at the hard-dough stage.

Year 2: Forage sorghum hybrid ADV 7232 (Alta Seeds, Irving, TX), was planted on June 22, 2020, at a seeding rate of 247,000 plants/ha, with 30.5-cm row spacing in seven irrigated acres. Fertilization followed soil test recommendations, and pest management included treatments for whorlworms and sugarcane aphids. Harvest occurred at the hard-dough stage using a Shredlage kernel processor.

Year 3: Grain sorghum hybrid Pioneer 845F (Pioneer Hi-Bred International, Johnston, IA) was planted on July 15, 2021, at 247,000 plants/ha in a twin-row configuration with 76-cm row spacing across 26 acres. Fertilization and pest management followed protocols similar to Year 2. Harvest was completed in October 2021 using a Shredlage processor.

Silage Processing and Storage: Harvested sorghum was chopped and treated with silage inoculants to enhance fermentation. The chopped forage was stored in 3-m (Year 1) or 3.65-m (Years 2 and 3) diameter plastic silage bags. The kernel processor roll gap was set at 1 mm to optimize starch digestibility.

Diet Composition: Heifers were fed total mixed rations (TMR) based on sorghum silage.

Year 1: Diet consisted of 89% sorghum silage, 10% dried distillers grains plus solubles (DDGS), and 1% vitamin-mineral premix (DM basis).

Year 2: Diet consisted of 89% sorghum silage, 10% high protein meal (cottonseed or carinata meal), and 1% vitamin-mineral premix.

Year 3: Diet consisted of 63% sorghum silage, 25% cotton gin byproduct, 10% cottonseed meal, and 2% vitamin-mineral premix.

Animal Management: Heifers were allocated to pens and fed ad libitum for 56 days in Years 1 and 2 and for over 100 days in Year 3. Initial and final body weights (BW) were recorded, and average daily gain (ADG), dry matter intake (DMI), and feed conversion ratio (F:G) were calculated. Feed cost of gain (FCOG) was determined based on ingredient prices and feed consumption.

Results

Table 1. Sorghum varieties utilized in each year, with their respective nutritive composition, cost and yield at harvest.

	YEAR 1	YEAR 2	YEAR 3
Hybrid	ADV7401	ADV7232	Pioneer845F
Type	Forage	Forage	Grain
Yield (fresh tonne/ha)	-- ⁴	57	38
DM ¹ , %	41.5	32	35
CP, % DM	10.7	10.6	8.4
NDF ² , % DM	49.8	47.8	32.1
TDN ³ , % DM	67.0	62.0	74.0
Starch, % DM	13.7	19.7	43.4
Cost, \$/tonne AF ⁴	-- ⁴	42	47
Cost, \$/tonne DM	-- ⁴	132	133

¹DM = dry matter

²NDF = neutral detergent fiber

³TDN = total digestible nutrients

⁴-- = not measured

Table 2. Performance response of backgrounding heifers fed a sorghum silage-based diet for 56d at the NFREC and economic calculation of the cost of gain in each of three years of data collection.

	Year 1	Year 2	Year 3
Initial BW ¹ , kg	329	331	318
Final BW, kg	367	390	391
DMI ² , kg/d	9.0	8.8	9.1
ADG ³ , kg	0.68	0.95	0.98
F:G ⁴ , kg feed DM/kg gain	14.0	9.2	9.3
FCOG ⁵ , \$/0.454 kg	0.90	0.61	0.63

¹BW = body weight

²DMI = dry matter intake

³ADG = average daily gain

⁴F:G = feed to gain ratio on a dry matter basis

⁵FCOG = feed cost of gain

Conclusions

Feed cost of gain (FCOG) decreased from \$0.90/0.454 kg in Year 1 to \$0.61/0.454 kg in Year 2, demonstrating improved efficiency with better crop yield and diet formulation. Year 3 maintained low FCOG despite incorporating cotton gin byproduct to reduce costs. Sorghum silage was shown to be a viable, cost-effective option for backgrounding beef heifers in the southeastern United States.

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Utilizing Legume Grass Mixtures for Baleage Production in the Coastal Plains Region: A Review of Research

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Keywords: Alfalfa, Bermudagrass, Red Clover, Baleage, Storage, Feeding

Bermudagrass (*Cynodon dactylon*) is one of the most prevalent warm-season perennial grasses grown in the Southeast US. Characterized by high yields and moderate at-best quality, bermudagrass has a substantial need for N fertilization and often-additional supplementation when fed to cattle. Alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*) are widely-used forage legumes throughout the United States that have a high relative forage quality (RFQ) and are a good source of digestible energy and protein; additionally, these legumes can fix nitrogen and achieve high yields without commercial nitrogen fertilizers. Often referred to as the forgotten legumes in the region, these legumes are re-gaining popularity with the development of new alfalfa and red clover varieties that are more tolerant to the tropical climate and insect pressure often associated with the Southeastern US. Growing these legumes with bermudagrass in a two species mixed sward can provide producers with dual benefit as interseeding into bermudagrass can reduce or eliminate the need for N fertilization, increase the relative forage quality by 30+ points, and decrease the need for additional supplementation when fed to livestock, as compared to monoculture bermudagrass alone. Furthermore, harvesting these mixtures as baleage can reduce the risk of losses and environmental impacts often associated with dry hay production in the region.

Research conducted over the past 9 years in Tifton, GA has evaluated the use of alfalfa-bermudagrass (**ABG**) and red clover-bermudagrass (**RBG**) mixed stands under baleage production. Alfalfa inclusion into bermudagrass stands increases the seasonal forage accumulation over bermudagrass fertilized with low to moderate rates of N fertilizer. In Tifton, GA, inclusion of alfalfa into bermudagrass increased seasonal dry matter (**DM**) forage accumulation by 13,000 kg ha⁻¹ in the second year as compared to BG fertilized with 84 kg N ha⁻¹ after each cutting (Hendricks et al., 2020). In this three-year study utilizing spring planted alfalfa, ABG mixtures were harvested 6 to 8 times from March to November, as compared to bermudagrass which was harvested only 4 times each year (Hendricks et al., 2020). After the establishment year, ABG nutritive value ranges from 180 to 247 g kg⁻¹ crude protein (**CP**) and 662 to 726 g kg⁻¹ total digestible nutrients (**TDN**). Differences in forage quality are mainly influenced by changing seasonal botanical composition and forage maturity at harvest (Hendricks et al., 2020).

While stand longevity can be a concern for producers, five-year-old alfalfa bermudagrass stands in Tifton, GA, have maintained over 30% alfalfa under various harvest strategies including baleage production, rotational grazing, and dual use management systems. In Headland, AL, and Tifton, GA, ABG mixtures planted in the fall of 2019 and harvested as baleage in 2020 and 2021 had increased seasonal herbage accumulation (14,752 and 16,350 kg DM ha⁻¹) as compared to a dual, cut and graze, strategy (5,662 and 7,663 kg DM ha⁻¹; Burt et al., 2024a). ABG baleage produced in Headland, AL, had average 56% of alfalfa in the stand, 211 g kg⁻¹ CP, and 621 g kg⁻¹ TDN, while baleage produced in Tifton, GA had on average 61% alfalfa in the stand, 218 g kg⁻¹ CP, and 652 g kg⁻¹ TDN (Burt et al., 2024a). In Tifton, GA, these stands continued to be productive into their third and fourth years, where average forage mass was 2068 and 3168 kg ha⁻¹ harvest⁻¹, respectively.

Additional work in Tifton, GA evaluated the impact of storage length on the nutritional value of baleage products. Pure (BG) and mixed (ABG) bales were evaluated at 4 timepoints throughout the storage period with cores collected pre-fermentation at initiation, and post-fermentation at 6 weeks, 9 months, and 12 months (Burt et al., 2024b). When compared between products, as expected ABG baleage had higher nutritive value at the initial 6-week sampling as compared to bermudagrass baleage. ABG baleage had 224 g kg⁻¹ crude protein and 656 g kg⁻¹ total digestible nutrients, while bermudagrass baleage had 147 g kg⁻¹ crude protein and 527 g kg⁻¹ total digestible nutrients. After 9 and 12 months of storage, both ABG and BG baleage had increased ADF concentration, but protein and digestibility remained similar to the initial values. A subset of bales were evaluated after storage for 24 months at which time significant change occurred within the bales. Conclusions from this work suggest that bale integrity is compromised the longer you store the bales, but if maintained throughout the storage process changes in nutritive value should be minimal (Burt et al., 2024b).

Forage preservatives and inoculants are available to producers to utilize in their operation; however, few studies have evaluated the effectiveness of these additives on legume bermudagrass mixtures in the southeast. Previously, Hendricks et al., 2021, evaluated various ferulic acid esterase producing microbial inoculants in a mini-silo trial in Tifton, GA. This evaluation was inconclusive on the potential benefits of these inoculants, as neither forage nutritive value nor in-vitro

digestibility parameters were improved with inclusion of the inoculant. Further work in Tifton, GA, is underway evaluating a propionic acid forage preservative and combination inoculant with ABG in mini silos (Stefancik et al., 2024). Preliminary data indicates that baleage treated with preservatives and/or inoculants did not differ in nutritive value from the untreated control; however, the ABG baleage produced was high quality with average 225 g kg⁻¹ crude protein and 787 g kg⁻¹ in-vitro true dry matter digestibility at 48 hours (Stefancik, et al., 2024).

While ABG mixtures can be harvested as hay or baleage, the chosen preservation method can affect cattle dry matter intake and rumen digestion parameters. A metabolism study completed at Auburn University found that pure alfalfa baleage had the highest dry matter intake, with ABG baleage (3.7 kg DM/hd/day) being intermediate between alfalfa baleage (8.0 kg DM/hd/day) and bermudagrass hay (2.9 kg DM/hd/day). As alfalfa inclusion increased, digestible crude protein increased. While alfalfa baleage had greater NDF and ADF digestibility, there were no differences between ABG baleage and bermudagrass hay. Additionally, rumen fluid metabolome diversity increased when the steers were fed baleage as compared to hay. Thus, inclusion of alfalfa, coupled with harvesting the forage as baleage, increased the steer's ability to utilize the forage nutrients and increased gut microbiome diversity.

While producers appreciate the perennial nature of alfalfa, it can be expensive to establish; therefore, producers are interested in alternative legumes, like red clover, which may be more economical (Burt et al., 2025). In Tifton, GA, red clover-bermudagrass or alfalfa-bermudagrass mixtures were harvested as baleage. ABG baleage had increased forage mass (1753 and 2158 kg DM ha⁻¹) as compared to red clover (1165 and 1946 kg DM ha⁻¹) in years 1 and 2, respectively (Doblado et al., 2022). While ABG had higher forage mass, forage nutritive value was similar regardless of legume species and averaged 174 g kg⁻¹ CP and 632 g kg⁻¹ TDN (Doblado et al., 2022). On-going work in Tifton, GA, is evaluating RBG forage accumulation in the spring to summer forage transition. Red clover establishment success varied based on location, and seasonal forage accumulation was decreased in red clover mixtures (Location 1: 2933 kg DM ha⁻¹; Location 2: 5721 kg DM ha⁻¹) as compared to N fertilized bermudagrass overseeded with annual ryegrass (Location 1: 3188 kg DM ha⁻¹; Location 2: 6759 kg DM ha⁻¹). Additionally, red clover botanical composition reached a maximum of 72% in May in Location 1; whereas in Location 2, red clover reached a maximum of 19% of the stand in April. While red clover mixtures have great potential in the southeast, further research evaluating the best methods for establishment and harvest management in differing environments is warranted.

Overall the addition of a legume component to bermudagrass swards results in a marked increase in seasonal yield and extended use of the land unit from summer into spring and fall. Further, the legume component eliminated the need for Nitrogen fertilizer while also increasing forage quality parameters CP and TDN and decreasing fiber factors ADF and NDF. Likewise, while annual rainfall varied among years, the utilization of baleage production resulted in the ability to maintain timely harvests to elevate quality through preservation and leaf retention. Producers in sub-tropical regions dominated by bermudagrass should strongly consider the incorporation of longer lasting legume species into their grass swards and utilization of baleage production to help combat the continually changing environmental impacts often seen in dry hay production.

Total Mixed Ration (TMR) to Enhance Dairy Productivity in Kolhapur, Maharashtra, India

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Keywords: Total Mixed Ration, silage, dairy productivity, milk yield, feed efficiency, NDDB

Introduction

Total Mixed Ration (TMR) is a scientifically formulated feeding strategy designed to optimize dairy production while maintaining animal health. Kolhapur, a prominent dairy hub in Maharashtra, India faces significant challenges related to seasonal green fodder scarcity, inconsistent nutrient intake, and escalating feed costs. Traditional feeding methods have contributed to suboptimal milk yields, lower fat and Solid-Not-Fat (SNF) percentages, and inefficient utilization of agricultural by-products. TMR includes feed ingredients such as silage, concentrate, dry fodder, etc. blended into a single, homogeneous mixture. It offers a promising solution by providing a balanced, consistent diet, which can enhance milk production, improve animal welfare, and increase economic returns for small and marginal farmers whose land holding capacity is less.

Materials and methods

The objective of this project was to do a pilot in TMR facility at Warananagar, Kolhapur, and assess its impact on milk yield, milk quality, and the economic viability for farmers. A controlled 28 day trial involving *Holstein Friesian (HF)* crossbred cows and *Murrah* Buffaloes was conducted to evaluate these parameters.

TMR Plant Setup:

- **Location:** Animal Holding Station, Warananagar, Kolhapur, India
- **Infrastructure and machinery:**
 - TMR mixer, Silage Baler, tractor with trolley etc
 - Office space for staff members
 - Animal shed
 - Two acres allocated for material storage
 - Existing covered structure utilized for plant operations

Feed Ingredients and Cost Analysis:

The TMR was composed of the following ingredients, with cost analysis detailed in the table below:

Table 1: TMR Composition and Cost Breakdown

Ingredient	% Inclusion	Total (kg)	Cost / kg (₹ / \$)	Total Cost (₹ / \$)
Concentrate	28%	1470	₹30.45 / \$0.3	₹44,761.5 / \$537.14
Dry fodder (wheat straw)	12%	630	₹12/ \$0.1	₹7,560 / \$90.72
Maize silage (30% DM)	59%	3097.5	₹7.8 / \$0.09	₹24,160.5 / \$289.93
Mineral mixture	0.4%	21	₹110/ \$1.3	₹2,310/ \$27.72
Bypass fat	0.4%	21	₹220 / \$2.6	₹4,620 / \$55.44
Buffer	0.2%	10.5	₹100/ \$1.2	₹1,050 / \$12.60
Salt	0.2%	10.5	₹35 / \$0.4	₹367.5 / \$4.41
Total	100%	5260.5	₹16.13 / \$0.19	₹84,829.5 / \$1,017.9

Table 2: Traditional Dairy Feed Composition and Cost analysis

Ingredient (with Category)	% Inclusion	Total (kg)	Cost/kg (₹ / \$)	Total Cost (₹ / \$)
Green Fodder – Napier Grass	62.32%	15	₹2.50 / \$0.03	₹37.50 / \$0.45
Dry Fodder – Sorghum / Maize husk	20.77%	5	₹9 / \$0.10	₹45 / \$0.50
Concentrate – Cattle Feed	16.62%	4	₹27.00 / \$0.38	₹108 / \$1.52
Mineral Mixture	0.29%	0.07	₹100.00 / \$1.20	₹7.00 / \$0.084
Total	100%	24.07	₹8.21 / \$0.106	₹197.50 / \$2.55

TMR Preparation Process:

1. All feed ingredients were precisely measured and mixed in a one-ton capacity mixer for uniform distribution.
2. The mixed feed was baled into 50-60 kg packages, with a shelf life of 60- 90 days.
3. Each bale was labelled with the manufacturing date and distributed to participating farmers for the trial.

Trial Design:

Animals: 5 HF crossbred cows, 2 Buffalo with a combined average baseline milk yield of 8.54 litres/day.

Duration: 28 days, with an initial acclimatization period of 7 days.

Data Monitoring: Daily measurements of milk yield, fat percentage, SNF content, and feed intake were recorded.

Results

The results of the trial showed significant improvements in milk quality across both HF crossbred cows and buffaloes.

Table 3: HF Crossbred Cows/Bufalo – Milk Yield performance, Quality and Price Comparison

HF crossbred Performance	Baseline Average for 7 Days	Week 1	Week 2	Week 3	Average (Week 1-3)	Change (%) (Baseline to average)	Milk Price Before (₹) / (\$)	Milk Price After (₹) / (\$)	Price % Change in total value
Milk yield (L/day)	10.36	10.43	9.44	10.21	10.02	-3.20%	₹29.8/L \$0.35/L	₹33.7/L \$0.39/L	9.38%
Fat (%)	3.88	4.17	4.12	4.2	4.16	7.30%	₹308.72	₹337.67	
SNF (%)	8.2	8.36	8.54	8.3	8.4	2.44%	\$3.58	\$3.92	
Buffalo Performance	Baseline Average for 7 Days	Week 1	Week 2	Week 3	Average (Week 1-3)	Change (%) (Baseline to average)	Milk Price Before (₹) / (\$)	Milk Price After (₹) / (\$)	Price % Change in total value
Milk yield (L/day)	6.72	7.1	7.79	7.73	7.54	12.15%	₹54.5/L \$0.63/L	₹62/L \$0.72/L	27.64%
Fat (%)	6.5	6.9	7	7.35	7.08	8.97%	₹366.24	₹467.48	
SNF (%)	8.4	8.93	9.1	9.25	9.09	8.23%	\$4.25	\$5.43	

Discussion: The trial results demonstrated several key benefits of TMR feeding:

Improved Milk Yield and Quality: Buffaloes exhibited a 12.2% increase in milk yield, with higher fat (8.97%) and SNF (8.23%) percentages, enhancing product value. HF crossbred cows showed an increase in fat (7.22%) and SNF (2.44%), but a slight decline in milk yield (-3.29%), likely due to dietary transition stress, adaptation period, or high baseline yield.

Economic Benefits: While the traditional feed cost was lower at ₹8.21 / \$0.106 per Kg, it had to be fed in significantly higher quantities to meet the animals' nutritional requirements. In contrast, TMR at ₹16.13/kg (\$0.19) provided a dense, balanced diet requiring nearly half the quantity for the same nutrient delivery. This not only reduced bulk handling and wastage but also improved feed efficiency. The higher milk prices from better fat and SNF levels, especially in buffaloes (27.6% value increase), made TMR more profitable despite its higher per kg cost.

Conclusions:

The pilot TMR initiative in Kolhapur successfully demonstrated the potential of scientifically balanced feeding in improving dairy productivity and profitability, particularly for small and marginal farmers. Buffaloes responded with notable gains in milk yield and quality, translating to a 27.6% increase in milk value. While HF cows showed modest milk quality improvements, a brief yield dip highlighted the need for longer adaptation. Overall, TMR's ability to deliver higher nutrient density with reduced feed volume makes it a scalable, sustainable solution. Widespread adoption across Maharashtra can help transform dairy farming into a more efficient and income-generating enterprise.

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Influence of Inoculated Corn-alfalfa Mixed Silage on Growth Performance of Hu Sheep

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Keywords: Alfalfa, Hu sheep, *Lentilactobacillus buchneri*, silage, whole corn

Introduction

Corn and alfalfa are the most important forages (cereal and legumes) in livestock production worldwide. While silage from whole corn is good in terms of fermentation and energy, silage from alfalfa is characterized by poor fermentation, low energy, and high nutrient loss. This study incorporates whole corn and alfalfa forage to complement each other's inadequacies. The addition of *Lentilactobacillus buchneri* as an inoculant can help to improve fermentation when silage is made during low temperatures and reduce silage quality losses during feed-out. Previous research (Ozturk et al., 2006) has demonstrated that mixing corn and alfalfa forages improves the quality of silage; however, the investigations are primarily small-scale laboratory experiments without any feeding trials. This study is therefore crucial to comprehending how the alfalfa ratio and inoculation affect the growth performance of Hu sheep.

Materials and methods

Ninety (90) male Hu sheep of 5 months of age weighing 29.27 ± 1.3 kg were randomly assigned to 6 dietary treatments in a Completely Randomized Design balancing for weight. There are 15 sheep in each treatment, split up into 3 pens (replicates), each with 5 animals. The sheep were given a total mixed ration consisting of 50% concentrate mix (corn grain, sugar beet pellet, and commercial concentrate) and 50% silage with or without *Lentilactobacillus buchneri* inoculation. The silage was composed of three proportions of whole corn to alfalfa forage at 0, 30, and 40% alfalfa inclusion. The experiment diets were iso energy (15.50 MJ/Kg DM ME) and crude protein (11.50%). With a 15-day adjustment period and a 90-day collection period, the study lasted for 105 days. Water was constantly available, and the animals were fed twice a day at 9 am and 4 pm leaving 5–10% as leftover. Experimental diets were formulated to meet the nutrient requirement of the lambs as recommended by feeding standards of meat-producing Sheep and Goats of Chinese agricultural industry standards (NY/T 816-2004) (Table 1). Data on feed intake was taken daily before the days' feeding. Body weight was measured at the beginning of the experiment and fortnightly thereafter. Utilizing the SPSS software (20.0; SPSS Inc., Chicago, USA), the gathered data was analyzed. A two-way ANOVA was used to determine the differences among the treatments at 0.05 level of significance and where means differ significantly, Duncan Multiple Range Test was used to separate means.

Results

Dry matter intake (DMI) was adversely affected by alfalfa inclusion, with treatments devoid of alfalfa exhibiting greater DMI. Similarly, inoculation with *Lentilactobacillus buchneri* decreased DMI in comparison to non-inoculated groups that had the same amount of alfalfa. The opposite is true in this study, which may be explained by the high fiber content (particularly NDF) of the alfalfa because it is the third cut, the bulkiness of silage with more alfalfa, and the larger amount of acetate in inoculated treatments, even though alfalfa addition is reported to improve acceptability and thus DMI (Wang et al., 2021). Sheep fed 40% alfalfa-based silage had a considerably higher average daily gain (ADG) than those fed sole corn and 30% alfalfa inclusion treatment. Increasing the amount of alfalfa in the diet increased feed efficiency (FE). Values were highest in the 40% alfalfa inclusion. Alfalfa's phenolic and other phytochemicals may have led to greater feed utilization. The trend in the FE is reflected in the final body weight. It increased with more alfalfa inclusion. Similarly, sheep given silage containing 40% alfalfa gained the most total weight (20.90 kg (NIT) and 21.37 kg (IT)). Treatment with 40% alfalfa resulted in a lower cost per kilogram of weight gain due to lower DMI and greater FE. The cost dropped from \$2.84 in 100% corn (NIT) to \$2.07 in 40% alfalfa (IT) based diets (Table 1).

Table 1: Effect of *Lentilactobacillus buchneri* inoculated whole corn - alfalfa mixed silage-based diets on growth performance of Hu sheep (n = 90)

Item	100% Corn		70% Corn + 30% Alfalfa		60% Corn + 40% Alfalfa		P value			
	NIT	IT	NIT	IT	NIT	IT	SEM	R	I	R x I
IBW (kg)	30.51	28.76	28.02	28.19	28.88	29.20	0.871	0.203	0.544	0.491
FBW (kg)	48.81 ^{abc}	45.5 ^{bc}	45.46 ^c	45.78 ^{bc}	49.77 ^{ab}	50.57 ^a	1.262	0.015	0.600	0.283
DMI (Kg)	1.55 ^a	1.45 ^b	1.35 ^{cd}	1.33 ^d	1.42 ^{bc}	1.37 ^{bcd}	0.025	<0.001	0.013	0.306
TWG (kg)	18.30 ^{bc}	16.74 ^c	17.44 ^c	17.59 ^c	20.90 ^{bc}	21.37 ^a	0.892	0.002	0.655	0.625
ADG (g)	203.36 ^{bc}	186.03 ^c	193.79 ^c	195.41 ^c	232.20 ^{ab}	237.45 ^a	9.517	0.001	0.665	0.448
FE (%)	13.06 ^c	12.72 ^c	14.07 ^{bc}	14.71 ^{abc}	16.35 ^{ab}	17.03 ^a	0.733	0.001	0.598	0.735
Daily Intake as fed (kg)	2.34	2.21	2.14	2.13	2.26	2.19	0.163	0.713	0.626	0.917
Cost of Feed Intake (\$)	0.58	0.55	0.52	0.51	0.52	0.51				
Cost of kg weight gain (\$)	2.84	2.96	2.71	2.63	2.18	2.07				

^{abc} = means with different superscript across rows are significantly different ($P < 0.05$), NIT= Non inoculated, IT= Inoculated, IBW=Initial Body Weight, FBW=Final Body Weight, DMI=Dry Matter Intake, ADG=Average Daily Gain, FE=Feed Efficiency

Conclusions

This study provided insight into the growth performance of Hu sheep fed mixed whole corn-alfalfa silage. The inclusion of alfalfa improved the performance of the sheep in terms of ADG and total weight gain at a reduced cost. Inoculation with *Lentilactobacillus buchneri* improved diet utilization significantly.

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Effects of GABA-enriched Alfalfa Silage on Dairy Goat Performance and Mammary Inflammatory Gene Expression

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Keywords: Gamma(γ)-aminobutyric acid (GABA), Dairy goats, mammary gland gene expression

Introduction

Gamma(γ)-aminobutyric acid (GABA), a known neurotransmitter, has been associated with several physiological functions. However, most animal studies with GABA feed it exogenously and mainly consider its effect on intake and homeostasis. We have recently found that inoculating silage with GABA producing lactic acid bacteria enriched the silage with GABA (Usman et al., 2024). Additionally, a human study reported that GABA significantly altered secretion of both pro- and anti-inflammatory types of cytokines. Dairy animals being vulnerable to oxidative stress and inflammatory disease (mastitis). We fed a GABA-enriched alfalfa silage to dairy goat and determined it effect on intake, milk yield and quality, and the expression of antioxidant related as well as pro- and anti-inflammatory genes in the mammary gland.

Materials and methods

The experiment was conducted for 45 days with 36 dairy goats with a mean body weight of 41.07 kg at mid-to-end lactation period. The goats were fed TMR containing uninoculated alfalfa silage (CK) for 10 days to determine dry matter intake (DMI) and milk yield, based on which they were blocked and randomly assigned to three dietary treatments (12 per treatment) using blockTools package in R studio. The dietary treatments were CK, TMR containing alfalfa silage inoculated with non-GABA producing *Lentilactobacillus buchneri* AH35 (AH35) and TMR containing alfalfa silage inoculated with GABA producing *Lent. buchneri* YM9 (YM9). Feeds were offered *ad libitum* (60% morning, 40% evening) after milking. Following a 14-day adaptation period, data was collected over 21 days, with milk samples obtained every 7 days, pooled (6:4 morning: afternoon), and preserved at 4 °C with bronopol for analysis. On the final day, mammary gland tissue samples were collected for the analysis of mRNA expression using qPCR. Mixed model was used for analysis, with diet treatments as fixed effects and animals as random effects.

Results

Dairy goats in the CK group had the highest DMI, followed by those in AH35, and lastly the YM9 group which has the least DMI ($P < 0.05$) (Table 1). The milk yield per day was higher in CK and YM9 group, but the milk yield per kg of DMI was highest in YM9 group, which implies that the feed utilization efficiency was better in goats fed TMR containing alfalfa silage inoculated with *Lent. buchneri* YM9. Hence, the GABA concentration in the YM9 silage enhanced the feed utilization efficiency.

Table 1: Performance and milk components

Item	Treatments			SEM	p-values
	CK	AH35	YM9		
DMI, kg/d	1.84 ^a	1.78 ^b	1.66 ^c	0.010	<0.001
Weight gain	4.31	5.91	5.95	0.83	0.291
Milk yield, kg/d	0.64 ^a	0.58 ^b	0.64 ^a	0.016	0.008
Milk yield, g/kg DM	350 ^b	331 ^b	383 ^a	9.210	<0.001
ECM, kg/d	0.38	0.42	0.45	0.049	0.648
ECM, g/kg DM	209	241	267	28.7	0.367
Fat, g/kg DM	9.78 ^b	12.5 ^a	13.4 ^a	0.334	<0.001
Protein, g/kg DM	12.5 ^b	14.4 ^a	15.2 ^a	0.365	<0.001
Lactose, g/kg DM	15.2 ^b	14.9 ^b	17.1 ^a	0.428	0.001
Casein, g/kg DM	10.5 ^b	11.9 ^a	12.8 ^a	0.305	<0.001
Total solid (TS), g/kg DM	39.7 ^c	43.8 ^b	48.0 ^a	1.130	<0.001
Solid not fat (SNF), g/kg DM	31.5 ^b	33.2 ^b	36.9 ^a	0.879	<0.001

The dairy goats in both AH35 and YM9 treatments had higher yield of milk fat, protein and casein per kg of DMI compared to those in the CK treatment ($P < 0.05$). However, the goats in the YM9 treatment had the yield of milk highest lactose, TS and SNF per kg of DMI ($P < 0.05$). Therefore, feeding dairy goats with GABA enriched alfalfa silage doesn't only improve the milk yield per DMI, but also the quality in terms of lactose, TS and SNF content.

Fig. 1 revealed that dairy goats fed TMR containing either of *Lent. buchneri* treatment (YM9 and AH35) had the relative mRNA expression of the oxidation-related genes (NOX4, TNF and IFNG) been significantly downregulated ($P < 0.05$). on

the antioxidant-related genes, the goats in the YM9 treatment had the relative mRNA expression of *CAT* and *GSR* been significantly upregulated ($P < 0.05$). Notably, *SOD3* and *HMOX1* were significantly upregulated in the CK group ($P < 0.05$).

The gene expression data revealed that YM9 treatment is most effective at reducing inflammation and oxidative stress. This is evidenced by the lowest expression of pro-inflammatory genes (*NOX4*, *TNF*, and *IFNG*) and the highest expression of key antioxidant genes (*CAT* and *GSR*), indicating enhanced redox balance. In contrast, the AH35 treatment moderately reduces inflammation and oxidative stress, with slightly elevated antioxidant responses compared to CK treatment. The goats in the CK group exhibit the highest oxidative stress and inflammatory markers alongside the weakest antioxidant defenses, with high *NOX4*, *TNF* and *IFNG*, as well as low *CAT* and *GSR* expression. However, the higher *SOD3* and *HMOX1* in the CK group is likely because their expression is induced in response to the elevated oxidative stress caused by the higher expression of the pro-inflammatory makers, while the reduced stress in AH35 AND YM9 diminishes the need for the activation of the *SOD3* and *HMOX1* in the treatments.

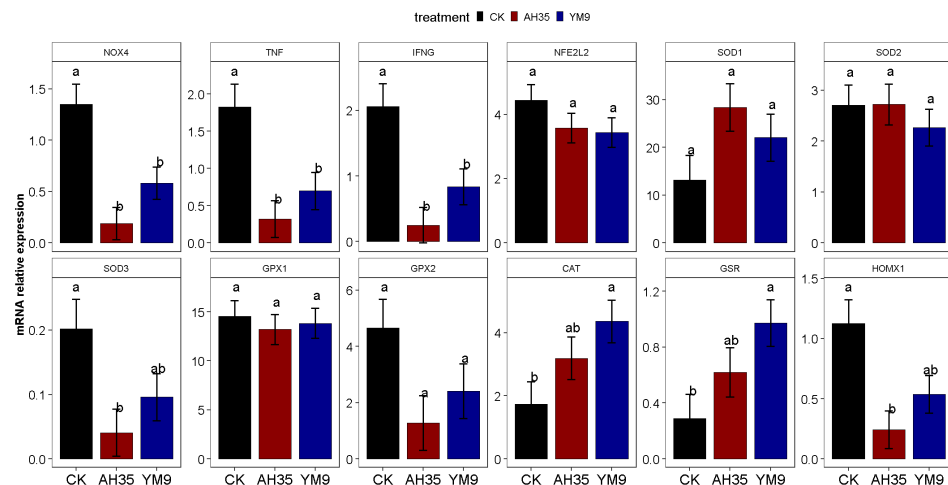


Fig. 1. Mammary gland genes expression. *NOX4*: NADPH oxidase 4; *TNF*: tumor necrosis factor; *IFNG*: interferon- γ ; *NFE2L2*: nuclear factor erythroid 2 like 2; *SOD1*: superoxide dismutase 1; *SOD2*: superoxide dismutase 2; *SOD3*: superoxide dismutase 3; *GPX1*, glutathione peroxidase 1; *GPX2*: glutathione peroxidase 2; *CAT*: catalase; *GSR*: glutathione-disulfide reductase; *HMOX1*: heme oxygenase 1.

Conclusions

Feeding dairy goats with a TMR containing alfalfa silage inoculated with GABA-producing *Lent. buchneri* YM9 improved feed utilization efficiency and milk quality, as evidenced by higher milk yield per kg of DMI and increased concentration of lactose, TS and SNF. Additionally, the YM9 treatment effectively reduces inflammation and oxidative stress while significantly boosting antioxidant defenses, as evidenced by the highest expression of *CAT* and *GSR* while downregulating the expression of pro-inflammatory genes.

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Bacteriocins in Silage Management: A Natural Solution to Spore-Forming Bacteria Challenges

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Keywords: Silage fermentation, Bacteriocins, *Clostridium*, Microbiota, Spoilage

Introduction

Contamination of silage by *Clostridium* causes unfavourable silage fermentation, spoiling, and financial losses in the dairy industry (Driehuis et al., 2018). Through silage making and preservation, the heat-resistant *Clostridium* spores continue to exist, causing silage quality deterioration and subsequent problems in cheese production (Avila et al, 2020; le Bourhis et al., 2007). Generally Recognized as Safe (GRAS) natural antimicrobial peptides called bacteriocins are becoming increasingly popular as efficient substitutes for chemical preservatives. By targeting particular bacterial populations, these bioactive chemicals prevent or eliminate undesirable microorganisms while preserving product quality (Soltani et al, 2021). The objective of this study is to determine the efficacy of three bacteriocins (Nisin, Enterocin, and Brevibacillin) as well as a combination of Nisin and Brevibacillin in reducing *Clostridium* population in silage and their impact on silage quality and nutritive value.

Materials and methods

Silage was prepared using third-cut alfalfa at the flowering stage. Forage was treated to produce silage liable to clostridial fermentation. The experiment was conducted in a randomized block design, with 4 experimental units per treatment. Forage was treated with saline (0.9% NaCl control), inoculated with clostridia, and then treated with bacteriocin. A consortia of clostridia inoculum (5 clostridial strains) was applied to the forage (4kg fresh materiel, FM) at 5 log CFU/g (FM). Bacteriocins applied at specified concentrations: Nisin (60 µg/g FM), Enterocin (15 µg/g FM), Brevibacillin (25 µg/g FM), and Nisin + Brevibacillin (7 µg/g FM). Forage was ensiled in experimental silos (1.7 L) in duplicates and was sampled at ensiling and 4, and 100 days post ensiling. Silage microbial population was determined by microbial count (total count, lactic acid bacteria (LAB), and *Clostridium*) and silage fermentation quality (lactic, acetic, and butyric acids) was quantified. Statistical analysis was conducted to determine treatment effects, using a two-way analysis of variance. The significance level was declared at $P < 0.05$ using Dennett's test.

Results

At Day 0, total bacterial and LAB (lactic acid bacteria) counts were similar across all treatments, averaging 8.0 log CFU/g and 5.0 log CFU/g, respectively, confirming effective inoculation and preparation. By Day 4, clostridia count in the untreated control silage averaged 6.5 log CFU/g. Treatments with Nisin and Nisin + Brevibacillin demonstrated significant reductions compared to the control, with clostridia counts reduced to 1.32 and 1.04 log CFU/g, respectively. Furthermore, clostridia were undetectable in silages treated with Brevibacillin and Enterocin (Table 1). On the other hand, the LAB population on Day 4 showed no significant differences across all treatments, indicating that bacteriocins did not negatively impact the LAB population. By Day 100, clostridia count in the untreated control silage remained elevated at 5.33 log CFU/g. In contrast, clostridia were completely undetectable in all silages treated with bacteriocins. Also, in silages spiked with clostridia inoculum, bacteriocin treatments significantly suppressed clostridia growth compared to the clostridia-contaminated control. Enterocin treatment showed the lowest remaining clostridia count of 3.5 log CFU/g. LAB populations at Day 100 were higher in all bacteriocin-treated silages compared to both the untreated control and the clostridia-contaminated control. Organic acid analysis revealed improved fermentation profiles in silages treated with bacteriocins. By Day 4, lactic acid concentrations in untreated silages (control) were 1.6 g/kg DM, and the silages treated by Nisin demonstrated a higher amount of lactic acid at 2.2 g/kg DM (Figure 1). In clostridia-spiked silages, bacteriocin-treated ones ranged from 1.2 g/kg DM for Nisin to 2.1 g/kg DM for the silages treated with Brevibacillin (Figure 2). Acetic acid concentrations on Day 4 showed no significant differences among bacteriocin-treated silages compared to control apart from the treatment which was spiked with clostridia and Brevibacillin where the amount reached 1.6 g/kg DM, compared to just 0.46 g/kg DM in untreated clostridia-spiked silages. Butyric acid, a spoilage indicator, was significantly lower in bacteriocin-treated silages. By Day 4, butyric acid concentrations in clostridia-spiked untreated silages reached 0.7 mg/kg DM, while bacteriocin-treated silages showed significantly reduced levels, with Brevibacillin-treated silage at 0.4 mg/kg DM. Similarly, in silages treated without clostridia inoculation, Brevibacillin-treated silage achieved a butyric acid concentration of 0.29 mg/kg DM compared to the untreated control at 0.34 mg/kg DM. By Day 100, treatments with Enterocin and the combination of Nisin + Brevibacillin achieved the highest lactic acid concentrations (nearly 4 g/kg DM) (Figure 1), indicating enhanced fermentation efficiency and superior preservation quality. Among clostridia-contaminated treatments, the silages treated with Nisin showed the highest concentration of lactic acid at 2.3 g/kg DM compared to the clostridia-spiked silages (Figure 2). The effects of bacteriocins were consistent in silages both spiked and unspiked with clostridia, demonstrating significant reductions in spoilage-associated metabolites and improvements in silage quality.

Conclusions

In conclusion, bacteriocin treatments significantly reduced clostridia counts, with some treatments rendering clostridia undetectable by Day 100, while simultaneously preserving or enhancing the LAB population and improving fermentation

profiles by lowering spoilage-associated metabolites such as butyric acid. The results highlight that all bacteriocins performed effectively in reducing spoilage indicators and supporting lactic acid production. These findings underscore the potential of bacteriocins as sustainable biopreservatives to mitigate silage spoilage and improve feed quality. Future work should focus on optimizing dosage and application under field conditions.

Table 1. Effects of Bacteriocin treatments on clostridia counts (log CFU/g) in silage at Day 0, Day 4, and Day 100.

Treatments	Clostridia Counts			SEM
	Day00	Day04	Day100	
Control	5.96	6.393	5.33	0.084
Nisin	2.11	1.32*	0*	0.140
Enterocin	1.76	0*	0*	0.054
Brevibacillin	1.95	0*	0*	0.011
Nisin+Brevibacillin	2.38	1.042*	0*	0.0335
Clostridia	6.908	7.948	6.8	0.042
Clostridia+Nisin	3.22	4.06*	4.33*	0.158
Clostridia+Enterocin	2.526	2.03*	3.5*	0.304
Clostridia+Brevibacillin	2.09	4.32*	5.26*	0.162
Clostridia+Nisin+Brevibacillin	2.75	2.035*	5.744*	0.226
* indicates significant differences compared to the untreated control at the same time point and clostridia-contaminated silage at the same time point (P < 0.05). SEM: standard error of means				

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Propionic Acid Effects on Nutritive Value and Fermentation Characteristics of Sunn Hemp Silage

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Keywords: sunn hemp, propionic acid, nutritive value, silage

Introduction

Sunn hemp (*Crotalaria juncea* L.) is a warm-season annual legume that has been widely used as a cover crop due to the rapid growth, potential to decrease soil nematode population, and ability to fix atmospheric N (Cook & Scott, 1998). Sunn hemp has also been used as forage for livestock due to acceptable forage quality (Garzon et al. 2021).

Silage is a commonly used forage conservation practice in tropical and subtropical areas due to excessive rainfall during the growing season. However, limited concentration of water-soluble carbohydrates (WSC) and high buffering capacity are unfavorable characteristics of warm-season legumes to produce silage with desirable fermentation characteristics. Therefore, it is necessary to explore management practices to improve fermentation and preservation of warm-season annual legumes.

Propionic acid is an effective antimycotic agent and has been used to inhibit undesirable microorganisms and improve aerobic stability of corn silage. Kung et al. (1998) observed that propionic acid was effective to reduce the heating in corn silage and total mixed rations. However, there are limited reports in the literature about the effects of propionic acid in warm-season legume silage. Some producers apply additives containing propionic acid and other antifungal components in the TMR prior to feeding; however, Kung et al. (1998) suggested that controlling yeast at the time of ensiling is more efficient than trying to control their numbers and metabolism in the feed bunk.

There is limited information in the literature about management practices to potentially improve sunn hemp silage fermentation and nutritive value. Therefore, the objective of this study was to evaluate the use of propionic acid to improve nutritive value and fermentation characteristics of sunn hemp silage.

Material and Methods

The research was conducted at the Range Cattle Research and Education Center, Ona, FL (27°31'N e 81°51'W) from May to November 2018. Fifteen experimental units (plots) of 'Ubon' sunn hemp were seeded in a prepared seedbed in May 2018 with a seeding rate of 25 kg ha⁻¹. Plots were 6 x 6 m with 2 m aisles between plots. Plots were harvested 8 wk after seeding at ground level, chopped to a 5-mm particle size, and placed in mini-silos. The mini-silos (PVC pipes with rubber caps with capacity of 1.5 kg of green forage) were filled immediately after harvest.

Treatments were 3 levels of propionic acid, 0, 0.5, and 1.0% of the forage green weight distributed in a randomized complete block design with 5 replicates. Propionic acid was applied with a hand sprayer before ensiling. Silos were opened 12 wk after ensiling and analyzed for nutritive value, fermentation characteristics and bacterial relative abundance.

Forage samples were ground in an Udy mill (Udy Corporation, Fort Collins, CO.) to pass a 2-mm screen. Samples of the ensiled forage were analyzed for DM, CP, NDF, ADF, in vitro true digestibility (IVTD), NH₃-N, pH, VFA concentrations, and yeast and mold counts (AOAC, 2005). In addition, genomic DNA was extracted using the Mag-Bind® Universal Pathogen 96 Kit (Omega Bio-Tek, Norcross, GA) in accordance with manufacture instructions.

Data was analyzed using Proc Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effect of treatment and the random effect of block. Orthogonal polynomial contrast was used to test treatment effects. The means reported were least square means and considered significant when $P < 0.05$.

Results

There was no effect of propionic acid levels on DM concentration ($P = 0.75$; Table 1), however, there was a linear increase in DM recovery with increasing levels of propionic acid ($P < 0.01$). In addition, increasing levels of propionic acid increased CP, and IVTD linearly, while decreasing NDF concentration ($P < 0.03$). There was no effect of propionic acid on ADF and NDFD concentrations ($P > 0.23$; Table 1).

There was a linear decrease in pH ($P < 0.01$), acetic acid ($P = 0.02$), butyric acid ($P < 0.01$), isobutyric acid ($P = 0.01$), ammonia ($P < 0.01$), and yeast and mold count ($P < 0.03$) with increasing levels of propionic acid (Table 2). Conversely, lactic acid and propionic acid concentration increased ($P < 0.01$) with increasing levels of propionic acid (Table 2).

Increasing propionic acid levels decreased the relative abundance of *Enterobacter* spp. (from 1.8 to 0.2%, $P = 0.03$, SE = 0.3) and *Clostridium* spp. (from 11.6 to 1.4%, $P = 0.02$, SE = 2.0), and increased the relative abundance of *Lactobacillus* spp. (from 6.7 to 0.6%, $P = 0.04$, SE = 0.2) and *Weissella* spp. (from 43 to 72%, $P < 0.01$, SE = 6.2)

Table 1. Nutritive value and DM recovery of sunn hemp silage treated with different levels of propionic acid.

Item	Propionic acid (%)			Contrast	SE
	0	0.5	1.0		
DM (%)	19	19	20	NS ¹	1.2
DM recovery (%)	96	98	98	Linear ²	0.5
CP, % DM	13.1	14.3	16.7	Linear	0.5
ADF, % DM	58.4	54.8	54.0	NS	1.7
NDF, % DM	66.6	63.2	61.7	Linear	1.7
IVTD, % DM	55	57	57	Linear	0.4
NDFD, % DM	47	48	49	NS	0.8

¹ NS = non significant

² Linear = $P \leq 0.05$

Table 2. Fermentation characteristics of sunn hemp silage treated with different levels of propionic acid.

Item	Propionic acid (%)			Contrast	SE
	0	0.5	1.0		
pH	6.1	5.4	4.5	Linear ¹	0.3
Lactic acid, % DM	0.1	1.4	5.6	Linear	0.7
Acetic acid, % DM	4.5	2.4	2.1	Linear	0.4
Propionic acid, % DM	1.6	2.4	4.0	Linear	0.3
Butyric acid, % DM	4.5	3.9	0.4	Linear	0.9
Isobutyric acid, % DM	0.6	0.4	0.1	Linear	0.1
Ammonia, % CP	52	42	21	Linear	2.5
Yeast (log cfu/g)	2.0	1.5	0.6	Linear	0.1
Mold (log cfu/g)	1.8	1.0	0.8	Linear	0.2

¹ Linear = $P \leq 0.05$

Discussion:

The addition of propionic acid decreased silage pH and the relative abundance of *Enterobacter* and *Clostridium*, which has limited tolerance to low pH. Fermentation of *Clostridium* leads to a breakdown of CP (Kung and Shaver, 2001) and the decreased activity of *Clostridium* resulted in silage with lesser ammonia and greater CP concentration.

The increase in lactic acid was likely driven by the increase in *Lactobacillus* and *Weissella* communities, which are responsible to convert water-soluble carbohydrates into compounds, such as lactic acid. Propionic acid has been effective to decrease mold and yeast count (Kung et al., 1998) and increase aerobic stability of silage. Greater IVTD was result of better fermentation profile and preservation of digestible components, such as non-structural carbohydrates.

Conclusions

Propionic acid treatment is an effective management practice to improve nutritive value and fermentation characteristics of sunn hemp silage.

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Impact of Selected Lactic Acid Bacterial Strains on Grass Silage Fermentation

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Keywords: grass, LAB, silage, DM loss, sampling

Introduction

An important objective of ensiling is to minimize nutrient losses and the formation of undesirable fermentation end products during the ensiling process, as these can reduce the nutritional value, hygienic quality, and palatability of the silage. The most common criteria for selecting an ideal silage inoculant includes its rapid growth in the ensiled material and its ability to outcompete the natural microbial flora (Saarisalo *et al.*, 2007). Fermentation processes using different strains on the same substrate can yield significant different results due to the unique properties of each substrate and selecting an appropriate silage inoculant for specific starting substrates is crucial (Amaral *et al.*, 2020). Our study evaluates the effects of newly selected strains of lactic acid bacteria (LAB) on the fermentation process of grass silage. The strains being investigated—*Enterococcus faecium* DSM 16567, *Lactococcus lactis* DSM 34583, and *Lentilactobacillus buchneri* DSM 34586—are compared to a control group of non-inoculated silage. This approach allows to assess the potential benefits of these specific LAB strains in improving the quality of silage, particularly by examining their impact on fermentation characteristics such as pH reduction, organic acid profiles, and preservation of nutrients.

Materials and methods

The silages were prepared from grass from grass wilted to a DM content of 294 g kg⁻¹ and containing 51 g kg⁻¹ DM of WSC. The grass was contaminated with epiphytic yeast, mold, and clostridia at levels of 5.69, 5.39, and 2.05 log cfu/g fresh grass, respectively, with a LAB count of 5.64 log cfu/g. The grass was chopped in the laboratory to a theoretical length of approximately 2-3 cm and then ensiled in 3-L mini-silos. The laboratory study on grass silages comprised four treatments: (C) Control, with no inoculant applied; (EF) treated with *Enterococcus faecium* DSM 16567; (LL) treated with *Lactococcus lactis* DSM 34583; and (LB) treated with *Lentilactobacillus buchneri* DSM 34586. All inoculants were applied at a rate of 100,000 CFU per gram of fresh grass. The silages were stored at room temperature (20.0 ± 1.0°C) for 90 days. Before applying the inoculants, chopped grass was sampled and analysed for DM, pH, buffer capacity, nitrate levels, nutrient composition, and microbial parameters, including LAB, yeast, mold, and *Clostridia* spp. At day 90 of storage, samples of the ensiled grass (five replicates for each treatment) were analysed for DM, nutrient content, fermentation parameters (including pH, ammonia-N, alcohols, 1,2-propanediol, lactic acid, and volatile fatty acids [VFAs]), and microbial characteristics (LAB, yeast, mold, and *Clostridia* spp.). The DM content was corrected for loss of volatile compounds during oven drying (Weissbach and Strubelt, 2008). Additionally, pH was measured on day 3 of the fermentation process. Each mini-silo served as the experimental unit for statistical analysis, which was conducted using the PROC GLM procedure in SAS 9.4 software. The model included treatment as the main fixed effect for each inoculant, with statistical significance defined at $P \leq 0.05$.

Results

The effects of inoculation were evaluated based on DM loss during fermentation, pH levels, metabolite profiles (including acids and alcohols), microbial populations, and nutrient composition of the silage. The treatment with inoculants resulted in a significantly higher ($P < 0.05$) DM content corrected for volatile content and a significantly lower ($P < 0.05$) DM loss compared to the C silage (Table 1). When compared to C, grass silage treated with LB demonstrated the lowest difference in DM loss, based on the average across different LAB strains ($P < 0.05$). Similar results were observed when analysing pH values ($P < 0.05$). The LB silage resulted in the highest ($P < 0.05$) concentration of 1,2-propanediol and the lowest ($P < 0.05$) lactic/acetic acid ratio compared with the C silage and the other two inoculant treatments. Additionally, all inoculant treatments (EF, LL, and LB) significantly reduced ammonia-N, alcohols, and butyric acid concentrations, as well as yeast and clostridia count, compared with the untreated silage (C).

Table 1. Fermentation characteristics of the grass silages untreated or inoculant treated

Item	C	EF	LL	LB
DM	263.3	275.9*	275.4*	264.2
DM corrected, g/kg	273.3	284.6*	284.3*	277.3*
DM loss, g/kg	81.0	39.50*	41.1*	67.0*
Crude protein, g/kg DM	188.3	196.3	197.3*	190.0
NDF, g/kg DM	517.5	515.7	513.3	516.0
ADF, g/kg DM	323.6	319.1	319.9	321.7
WSC, g/kg DM	2.34	3.00	2.54	2.40
pH at day 3 of fermentation	4.92	4.80*	4.76*	4.88
pH at day 90 of fermentation	4.38	4.14*	4.18*	4.36
Ammonia-N, g/kg total N	56.74	35.60*	34.46*	45.24*
Alcohols, g/kg DM	7.50	4.38*	4.08*	6.16*
Propanediol 1,2, g/kg DM	0.28	0.20	0.22	1.64*
Lactic acid, g/kg DM	66.80	94.20*	98.26*	63.88
Acetic acid, g/kg DM	28.22	22.30*	23.26*	41.70*
Butyric acid, g/kg DM	0.62	0.18*	0.18*	0.42*
Lactic/acetic acids ratio	2.38	4.28*	4.23*	1.52*
Propionic acid, g/kg DM	0.40	0.34	0.34	0.92*
Lactic acid bacteria, Log ₁₀ CFU/g	7.58	8.20*	8.17*	8.46*
Yeasts, Log ₁₀ CFU/g	1.86	1.34*	1.26*	1.12*
Mold, Log ₁₀ CFU/g	1.42	1.18	1.18	1.12
Clostridia, Log ₁₀ CFU/g	1.66	1.46*	1.32*	1.36*

C–non-inoculated, EF- inoculated with *Enterococcus faecium* (DSM 16567), LL- inoculated with *Lactococcus lactis* (DSM 34583), LB–inoculated with *Lentilactobacillus buchneri* (DSM 34586) * – describe significant differences between control and separate treatments at $P < 0.05$

Conclusions

The tested LAB strains effectively reduced the formation of undesirable fermentation products such as ammonia-N, alcohols, and butyric acid, while also suppressing yeast growth. Although the *Lentilactobacillus buchneri* DSM 34586 strain was less effective in reducing pH and DM loss, it produced higher concentrations of acetic acid and 1,2-propanediol.

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Effects of Microbial Inoculation on Silage Fermentation and Preservation Characteristics of Cool-Season Grass-Legume Mixtures in Southeastern Dairy Systems

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Keywords: Cool season forages, microbial inoculation, grass-legume mixtures

Introduction

Cool-season forages play a critical role in southeastern dairy systems, providing high-quality feed during winter. Southeastern dairy producers have benefited from forage variety development on their dairies, prior to cultivar release. The outcome has resulted in over 14 new recommended forages for southeastern dairies, including 5 oats, 2 cereal ryes, 3 triticale and 4 ryegrass cultivars (Wallau et al., 2024). The lack of suitable 'non-grass' annual forage candidates to help reduce pest cycles while providing ample, high feed value for dairies, continue to challenge producers. Annual cool-season legumes have not been adopted by area dairy producers, even though they are interested in such options. Seed cost, limited seed availability and limited information of potential utility in their systems are concerns worth considering. Grass-legume mixtures offer opportunities to enhance forage productivity and silage preservation characteristics, ensuring a stable, nutritious feed supply for dairy herds during critical periods (Dewhurst, 2013). We aimed to evaluate the effects of preservation characteristics with and without microbial inoculation, and nutritive value, of silage made from cool-season grass-legume mixtures grown under southeastern dairy conditions. We hypothesized that silage prepared from cool-season grass-legume mixtures will demonstrate superior nutritive value and microbial inoculation will further enhance preservation characteristics.

Materials and methods

This study was conducted at the University of Florida (UF) Dairy Research Unit. Cereal rye (FL405) or triticale (Trical342) were combined with winter pea (Icicle) and hairy vetch (AU Merit) in 100:0 and 50:50 ratios, resulting in six forage combinations, which were planted in four replicate blocks. The forages were sown in the last week of October on an irrigated field at the UF Dairy Research Unit in Hague, FL, and harvested in the last week of March 2024. They were then wilted to 40% dry matter for ensiling. Wilted forage from each block was divided into two 10-kg piles, which were randomly assigned to one of the following treatments: 1. **Control (CON):** Forage combinations ensiled without inoculant. 2. **Inoculant (INOC):** Forage combinations ensiled with the recommended dose of an inoculant blend containing *Pedococcus pentosaceus* NCIMB 12455 and *Lactiplantibacillus plantarum* NCIMB 12422, along with enzymes. The inoculant was applied at 2 g/ton of fresh forage (10^5 CFU/g) by dissolving it in 450 mL of deionized water and spraying it in a fine mist onto one pile from each plot while mixing continuously. For the control silages, an equivalent amount of distilled water was applied. Treated forages were packed into two vacuum-heat sealed nylon-polyethylene standard barrier bags (0.09-mm thickness, 40 × 61 cm; Doug Care Equipment Inc.), with air removed before sealing using an external clamp vacuum pump (Bestvac; distributed by Doug Care Equipment Inc.). The vacuum-sealed bags were ensiled at room temperature (23°C) for 30 and 90 days, with four replicate bags prepared for each treatment combination. Data analysis was performed using the GLIMMIX procedure in SAS, with the model including the fixed effects of forages, microbial inoculation, and their interaction and results were analyzed separately for 30 and 90 d. Statistical significance was set at $P < 0.05$, while a tendency was noted when $0.10 > P > 0.05$.

Results

Microbial inoculation decreased silage pH (Forage x INOC; $P = 0.03$) and increased lactic acid concentrations (Forage x INOC; $P = 0.04$) after 30d of ensiling for every forage combination except cereal rye. No effects were observed on silage pH with microbial inoculation after 90 d ensiling duration ($P > 0.05$); however, lactic acid concentrations were greater for the combination of Triticale x Winter pea compared with Rye and Rye x Winter pea combination (Forage; $P = 0.01$). No treatment effects were observed on acetic acid concentrations after 30 d ($P > 0.05$); however, a tendency towards greater acetic acid concentration was observed with Rye x Vetch and Rye compared with other forages after 90 d ensiling duration (Forage; $P = 0.08$). Dry matter loss was lower with INOC for every forage combination however DM loss increased with INOC for Rye (Forage x INOC; $P = 0.05$) after 30 d ensiling. No treatment effect was observed on DM loss after 90 d ensiling.

Conclusions

In conclusion, the use of microbial inoculation improved silage preservation characteristics during the early stages of ensiling, as indicated by reduced pH and increased lactic acid concentrations after 30 d of ensiling, particularly for most forage combinations except cereal rye. However, these effects were not sustained after 90 d of ensiling duration. Lactic acid concentrations were notably higher in the triticale-winter pea combination after 90 days, indicating that specific grass-legume mixtures may exhibit superior fermentation profiles. Although no significant effects of inoculation on acetic acid concentrations were observed at 30 d, a tendency for greater acetic acid was noted in rye and rye-vetch combinations at

90 d, suggesting a possible shift in fermentation dynamics over prolonged storage. Dry matter losses were generally lower with inoculation for most forage combinations at 30 d, except for rye, which exhibited increased losses under inoculation treatment. However, no significant differences in dry matter loss were observed at 90 days. Overall, these results suggest that cool-season grass-legume silages can serve as viable high-quality feed options for dairy producers in the southeastern U.S., with microbial inoculation playing a role in enhancing early fermentation and reducing dry matter losses in specific forage combinations.

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Insights from Corn and Sorghum Variety Trials – 15 Years of Data

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Keywords: hybrid selection, hybrid trials, agronomics

Introduction

Whole-plant silage crops are relevant worldwide associated to their use as feed in livestock system (Ferraretto et al., 2018) and for biofuel industries (Tari et al., 2013). Corn, sorghum and sorghum x sudan (SS) hybrids are the most important silage crops (Getachew et al., 2016; Pupo et al., 2022). These crops have higher energy concentration than other tropical and subtropical forages and roughages, being widely used in the dairy sector (Ferraretto et al., 2018). The high energy concentration explains the high costs and value of these crops, that can reach over \$2.5 billion in the US. In Florida, NASS (2025) estimates 10,000 hectares of corn silage are planted annually to partially support a \$0.5 billion industry with 98,000 milking cows. Some of those hectares are covered with sorghum as a summer silage crop, which is considered a secondary crop to corn. Sustaining yield and profitability over time is crucial for operations including silage crops, where the choice of varieties or hybrids has an important effect on productive, economic, and environmental outcomes. In this context, variety trials provide essential information for farmers' decision-making, with high local and regional impacts. The objectives of this study were to summarize long-term data assessing silage yield, nutritive value, and estimated milk production of corn, forage sorghum, and SS planted in spring and summer and test differences in species and planting season in a tropical environment.

Materials and methods

Agronomic trials for corn and sorghum hybrids (commercial and experimental) targeting silage production have been carried out between 2008 and 2023 at the Plant Science and Research Education Unit of the University of Florida in Citra, Florida. Over this timeframe, 959 entries were tested (669 corn, 202 sorghum, and 88 SS), planted in two seasons (Spring and Summer). Parameters measured included biomass production, nutritive value (fiber components, starch, sugars, digestibility, protein, etc.), and estimated milk production (Diepersloot et al., 2024). Agronomic and estimated milk production responses were evaluated across planting seasons using linear mixed models. Fixed effects included species and planting season, while hybrids and year were random effects. In addition, a temporal stability index was estimated as μ/σ , where μ is the mean for the 15-year period, and σ is the temporal standard deviation over the same interval, for the combination of species and planting season. Lastly, a decision tool integrating planting dates and relative maturity was developed to guide management under varying climatic scenarios.

Results

Corn planted in spring outperformed other species, regardless of planting season. Its yield was 10% and 27% greater than SS and forage sorghum planted in spring. Corn and SS planted in the summer produced 5.9 and 6.1 Mg ha⁻¹ DM less than spring plantings, whereas forage sorghum decreased by 2.3 Mg ha⁻¹ DM. Corn silage generally showed the best nutritive value, followed by forage sorghum and SS. Additionally, corn planted in spring showed a slightly superior nutritive value than summer corn, whereas this difference was smaller for forage sorghum and SS. The milk production per ha, estimated as the combination of DM yield and nutritive value, of corn planted in spring was 50% greater than corn planted in summer and forage sorghum planted in spring. Furthermore, milk production per ha 65% greater than SS planted in spring. The lowest milk production was for SS planted in summer.

For corn, there was no relationship between relative maturity (estimated days to black layer) and length of the growing season. Still, longer seasons resulted in greater yields associated with greater solar radiation intercepted. Yield stability across years for spring-planted corn was 78% greater than summer-planted corn (11.6 vs. 6.5, respectively), and 97 and 71% greater than spring-planted sorghum (5.9) and sorghum x Sudan (6.8). These latter species showed 48% greater stability when planted in spring than in summer (4.0 and 4.6 for sorghum and sorghum x Sudan, respectively).

Table 1 Silage agronomic responses and milk production per ha given the combination of species (corn, forage sorghum, sorghum x Sudan) and planting season (spring and summer). Crude protein (CP), neutral detergent fiber (NDF), digestible NDF (dNDF), acid detergent fiber (ADF) and starch are reported in proportion (%) of total dry matter (DM). Net energy for lactation (NEI) is reported relative of 1 kg DM.

	Corn		Forage sorghum		sorghum x Sudan		SE
	Spring	Summer	Spring	Summer	Spring	Summer	
Yield (Mg ha ⁻¹ DM)	19.2 ^a	13.3 ^d	15.1 ^c	12.8 ^d	17.5 ^b	11.4 ^e	0.4
CP (%)	8.0 ^{ab}	8.2 ^a	7.0 ^c	7.9 ^b	6.8 ^c	7.9 ^{ab}	0.2
NDF (%)	40.8 ^C	40.6 ^C	54.6 ^B	55.1 ^B	61.1 ^A	59.9 ^A	0.8
dNDF (%)	57.7 ^a	52.2 ^b	44.7 ^c	45.7 ^c	44.6 ^c	46.1 ^c	1.1
ADF (%)	23.5 ^d	22.8 ^d	33.7 ^c	34.0 ^c	40.7 ^a	38.0 ^b	0.6
Starch (%)	35.0 ^A	33.6 ^A	17.3 ^B	15.6 ^B	13.0 ^C	9.9 ^C	0.9
NE _i (MCal kg ⁻¹ DM)	1.61 ^a	1.56 ^b	1.41 ^c	1.42 ^c	1.26 ^d	1.28 ^d	0.02
TDN (%)	72.2 ^a	70.4 ^b	60.2 ^d	61.1 ^c	55.2 ^e	56.6 ^e	0.5
Milk (Mg ha ⁻¹)	64.2 ^a	42.4 ^b	41.3 ^{bc}	34.9 ^d	39.1 ^c	26.1 ^e	2.3

Means sharing a common lowercase letter are not statistically different among species and planting season (Tukey at $\alpha = 0.05$). Means sharing a common uppercase letter are not statistically different among planting seasons (Tukey at $\alpha = 0.05$). SE: standard error of the mean.

Conclusion

Corn is still the most valuable crop for silage in the Southern US. Despite the lower yield stability, sorghum is 55-80% cheaper growing option compared to corn, and often the choice for dry and growing cattle, because of the lower energy concentration. Considering the outcome difference (Milk; Table 1), the relative cost per unit of milk is even between the two options when the production cost of sorghum is 65% of the cost of corn in the spring or 82% for the summer. Hybrid choice remains an important decision for silage making: the top hybrids (95% percentile) result in about 30% greater yield and milk production compared to the average. In 2022, our team created a simple web tool to distribute the data to producers and practitioners, containing a summary of the data from the hybrid trials (<https://devfel.github.io/forage-hybrid-table/>). Last year (2024), there were 536 accesses (up from 140 accesses in the first year), heavily concentrated before planting season for corn between Jan 1 and Mar 15 (48%). This tool helps producers and industries in Florida, Georgia, and other surrounding states. Through direct surveys with corn producers who directly reported using data from our variety trials to make decisions on hybrid selection, representing about 20% of the total corn silage area in the state, this tool adds \$9M in value to the dairy industry, considering a gain of about 9,7 Mg ha⁻¹ of milk at a price of \$0.457 kg⁻¹ of milk.

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Are Whole-crop Silages of Cereals and Cereal-legume Intercrops a Good Alternative for Maize Silage?

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Keywords: whole-crop silage, fermentation, nutritional value

Introduction

The cultivation of silage maize is becoming more and more challenging over the years in several regions, due to extremer weather conditions (ranging from wet and cold periods to warm and dry periods), more stringent fertilization regulations, reduced availability of chemical herbicides, herbicide-resistance of weeds, etc. Whole-crop silage (WCS) of cereals in pure stand or intercropped with legumes are interesting candidate-alternatives for multiple reasons: 1) These crops are harvested earlier in the season than whole-crop maize, making them less prone to heat and/or drought stress during summer, 2) For intercrops with legumes, fertilization can be reduced thanks to the nitrogen fixing ability of legumes, 3) Cereals (whether or not intercropped with legumes) form a denser crop than maize, reducing weed development and thus also the need for herbicides, etc. To evaluate their potential, it is crucial to gain more insight in the silage fermentation characteristics and in the nutritional value for ruminants.

Materials and methods

In 2023, an ensiling trial with microsilos (volume of 2.75 liter; n=4) was performed with different winter crops (Table 1) harvested as WCS. At silo opening after 90 days, pooled samples per treatment were taken for determination of fermentation characteristics and nutritional value (cfr. Van den Bossche *et al.*, 2023).

Table 1. Treatments included in ensiling trial with whole-crop silage of cereals and cereal-legume intercrops.

Trt	Crop(s)	Cultivar(s)	Sowing density	Harvest date
1	barley	HB SY 220061 VGOLD585 ¹	180 seeds/m ²	June 15 th , 2023
2	barley	SU Hylona ²	180 seeds/m ²	June 15 th , 2023
3	barley-pea	HB SY 220061 VGOLD585 – Flokon ³	130-40 seeds/m ²	June 15 th , 2023
4	barley-pea	HB SY 220061 VGOLD585 - Flokon	100-50 seeds/m ²	June 15 th , 2023
5	triticale-pea	RGT Eleac ⁴ – Asteroid ³	130-50 seeds/m ²	June 21 st , 2023
6	triticale-pea	Lumaco ⁵ – Asteroid	130-50 seeds/m ²	June 21 st , 2023
7	triticale	RGT Eleac	350 seeds/m ²	July 4 th , 2023
8	triticale	Lumaco	350 seeds/m ²	July 4 th , 2023
9	triticale	Kasyno ⁶	350 seeds/m ²	July 4 th , 2023
10	triticale	Temuco ⁷	350 seeds/m ²	July 4 th , 2023
11	triticale-faba bean	RGT Eleac – Tundra ²	175-20 seeds/m ²	July 4 th , 2023
12	wheat-faba bean	Extase ⁴ – Tundra	130-20 seeds/m ²	July 4 th , 2023
13	méteil mixture	Proti Méteil ⁴	120 kg/ha	July 4 th , 2023
14	méteil mixture	own mixture ⁸	120 kg/ha	July 4 th , 2023

¹Syngenta, ²Limagrain, ³Agri-Obtentions, ⁴Jorion Philip Seeds, ⁵Arvesta, ⁶Danko, ⁷Lantmännen, ⁸Oats cv. Vodka⁴, rye cv. KWS Serafino⁵, triticale cv. Lumaco⁵ and pea cv. Flokon³ (resp. 75-50-50-45 seeds/m²).

Results

The fermentation characteristics and nutritional value parameters of the different WCS are presented resp. in Table 2 and Table 3. In line with large differences in dry matter (DM) content (range 301 - 555 g kg⁻¹ FM), the fermentation characteristics were highly variable between treatments. The pH values ranged from 4.21 to 5.12, showing a good acidification. However, ammonia fractions were in general quite high (range 8.6 - 19.8 %) and high values for butyric acid and alcohols (resp. range 0.2 - 26.4 and 3.0 - 37.3 g kg⁻¹ DM) were detected too, indicating a sub-optimal silage fermentation process (McDonald *et al.*, 1991). Also for the nutritional value parameters, large differences were found between treatments.

From a farmer's perspective, Net Energy for Lactation (NEL) and true protein digested in the small intestine (DVE; Tamminga *et al.*, 1994) are the most important parameters for ration calculation. Taking whole-crop maize silage with an average NEL value of 6.89 MJ kg⁻¹ DM and an average DVE content of 53 g kg⁻¹ DM (De Brabander, 2012; Eurofins, 2023) as a reference, the cereal and cereal-legume WCS perform less well. From the tested treatments, the highest NEL values were obtained for triticale in pure stand.

Table 2. Fermentation characteristics: pH, ammonia fraction (NH₃-N/total N), lactic acid (LA), acetic acid (AA), butyric acid (BA), alcohols (Alc.), sum of fermentation products (FP) and ratio of lactic acid to acetic acid (LA/AA).

Trt	Crop	DM	pH	NH ₃ -N/total N	LA	AA	BA	Alc	FP	LA/AA
		g kg ⁻¹ FM		Ratio	g kg ⁻¹ DM					
1	barley	433	4.44	8.76	29.8	6.18	4.58	28.9	69.4	4.82
2	barley	421	4.85	8.60	9.34	2.28	8.03	37.3	56.9	4.10
3	barley-pea	387	4.67	11.46	12.1	2.44	17.9	31.3	63.7	4.95
4	barley-pea	377	4.66	10.76	17.9	2.43	14.9	32.6	67.8	7.36
5	triticale-pea	315	4.35	15.31	34.5	5.77	26.4	10.5	77.1	5.97
6	triticale-pea	301	4.21	15.09	63.9	9.96	2.84	5.54	82.2	6.42
7	triticale	555	4.97	12.18	7.79	3.70	3.46	13.8	28.7	2.11
8	triticale	521	5.12	15.51	8.48	2.25	6.19	13.1	30.0	3.77
9	triticale	473	4.91	19.81	13.9	2.28	10.5	16.3	43.0	6.08
10	triticale	524	5.01	14.38	11.6	1.96	5.57	12.7	31.8	5.92
11	triticale-faba bean	468	5.02	15.95	8.14	2.23	9.57	16.4	36.3	3.65
12	wheat-faba bean	497	5.07	17.21	8.86	2.59	8.16	10.2	29.8	3.42
13	méteil mixture	423	4.43	11.06	40.1	8.16	0.17	3.02	51.5	4.92
14	méteil mixture	485	4.62	9.19	20.1	3.23	11.7	4.98	40.1	6.23

Table 3. Nutritional value parameters: crude protein (CP), starch, crude fiber (CF), fermentable organic matter (FOM), true protein digested in the small intestine (DVE), net energy for lactation (NEL), rumen-degradable protein balance (OEB), rumen-undegradable protein (RUP) and rumen-undegradable starch (RUS).

Trt	Crop	CP	Starch	CF	FOM	DVE	NEL	OEB	RUP	RUS
		g kg ⁻¹ DM					MJ kg ⁻¹ DM		%	
1	barley	85	380	224	498	44	5.44	-21	32.8	19.4
2	barley	79	275	243	517	42	5.51	-26	30.7	20.7
3	barley-pea	70	274	223	532	41	5.77	-32	28.4	19.6
4	barley-pea	84	235	253	517	43	5.44	-22	29.9	20.1
5	triticale-pea	113	110	325	498	42	4.83	6	25.6	21.3
6	triticale-pea	122	139	319	525	48	5.02	9	25.0	18.6
7	triticale	76	357	258	537	41	5.27	-28	27.3	12.0
8	triticale	72	298	277	514	36	5.01	-28	28.3	12.6
9	triticale	83	247	243	560	43	5.71	-22	19.1	31.5
10	triticale	85	248	256	574	46	5.65	-24	21.1	29.8
11	triticale-faba bean	80	277	254	539	40	5.41	-23	25.1	12.3
12	wheat-faba bean	72	277	268	527	37	5.21	-28	19.1	34.6
13	méteil mixture	142	130	323	503	55	4.81	16	31.8	26.1
14	méteil mixture	85	175	290	521	43	5.25	-23	27.5	32.9

Conclusions

From a nutritional point of view for ruminant feeding, whole-crop silages of cereals and cereal-legume intercrops can't live up to the high standards set by whole-crop maize silage. Still, there are possibilities towards optimization of the silage fermentation process (e.g. by silage additive application), which might have a beneficial effect on the nutritional value of cereals and cereal-legume intercrop silages.

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Effects of *Lentilactobacillus buchneri* and Papain on the Fermentation Profile, Protein Composition, and Protein Secondary Structure of High-Moisture Corn Silage

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Keywords: High-moisture corn, prolamin, protein composition

Introduction

Hydrophobic proteins in the corn endosperm are major factors limiting starch digestion in ruminants. These proteins form a starch-protein matrix with starch granules, creating physical and chemical barriers that hinder digestion and energy absorption in livestock. Glutelin (GLU) and zein, the primary hydrophobic protein components in the endosperm, are tightly bound to starch granules or surround them via hydrogen bonds and other interactions. This reduces microbial access to starch in the rumen, negatively impacting the production performance of ruminant animals. High-moisture corn (HMC) has recently gained popularity as an energy feed on Chinese farms due to its multiple benefits. It minimizes drying losses, increases grain yield, and improves net energy for milk production by 5–10% when included in dairy cow diets. To further enhance its utility, this study aims to break down the starch-protein matrix during anaerobic fermentation through the inoculation of targeted additives, facilitating efficient resource utilization and promoting sustainable agricultural practices. This research focuses on enhancing the degradation efficiency of hydrophobic proteins in HMC by inoculating *Lentilactobacillus buchneri* and papain. Additionally, it investigates the synergistic effects of microbial and enzymatic fermentation in optimizing HMC processing and improving nutrient utilization efficiency.

Materials and methods

The corn used in this study was cultivated at the experimental station of the Hebei Academy of Agriculture and Forestry Sciences and harvested on October 15, 2022. After harvest, the corn ears were threshed and crushed. Four treatment groups were established: Control group (CON); *L. buchneri* group (LB), with 1×10^6 cfu/g FW; Papain group (PAP), with 600 mg/kg FW; Combined addition group (LBPAP), with 1×10^6 cfu/g FW of *L. buchneri* and 600 mg/kg FW of papain. The pH, ammonia nitrogen (AN), lactic acid (LA), acetic acid (AA) and propionic acid (PA) contents of the HMC were analyzed. GLU and zein were sequentially extracted, and their contents were determined. Neutral detergent-insoluble protein (NDIP) content was measured following the method described by Licitra. Fourier-transform infrared (FTIR) spectra were recorded using a Thermo Fisher iS5 spectrometer.

Results

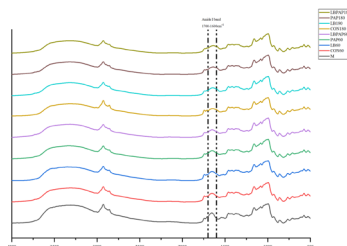
Table 1 shows the changes in the fermentation profile, AN, and protein composition of HMC silage after fermentation. The interaction between fermentation time and additives significantly affected pH, LA, AA and PA contents ($P < 0.05$). After 180 days of fermentation, PA content was significantly higher ($P < 0.05$) in the PAP and LBPAP treatment groups compared to other treatments, with the LBPAP group exhibiting the highest ($P < 0.05$) LA content. The interaction between fermentation time and additives also had a significant effect on zein and GLU content ($P < 0.05$), but no significant effect on AN and NDIP content ($P > 0.05$). Compared to 60 days of fermentation, 180 days significantly reduced zein and GLU contents ($P < 0.05$). Regarding AN and NDIP, fermentation for 180 days increased AN content ($P < 0.05$) and reduced NDIP content ($P < 0.05$), with the LBPAP treatment group showing the highest AN content ($P < 0.05$).

The FTIR spectra (Figure 2) revealed significant differences in the absorption peak intensity of the amide I region ($1700\text{--}1600\text{ cm}^{-1}$) before and after fermentation. Post-fermentation, the absorption peak broadened and its intensity decreased. Within the same treatment, the absorption peak intensity at 180 days was lower than at 60 days, with the LBPAP180 group showing the lowest intensity.

Table 1. Fermentation profile and protein composition of high-moisture corn silage

Ensiling day	Treatment	pH	LA	AA	PA	AN	NDIP	Zein	GLU
60	CON	3.92bc	1.61c	0.46bc	0.79c	2.94	2.56	4.11a	2.14ab
	LB	3.93bc	1.64c	0.45bc	0.83c	2.48	2.35	4.05a	2.27a
	PAP	3.89c	1.69c	0.51ab	1.02c	3.43	1.14	4.09a	2.03b
	LBPAP	3.89c	1.69c	0.55a	1.13c	4.42	2.07	3.87a	1.81c
180	CON	3.93bc	2.08bc	0.39cd	3.60b	4.81	2.09	3.27b	1.46d
	LB	3.95ab	2.54ab	0.35d	4.01b	4.93	1.11	3.44b	1.47d
	PAP	4.00a	2.37b	0.38cd	5.07a	4.53	1.19	3.20b	1.42d
	LBPAP	3.94bc	2.94a	0.34d	5.53a	5.91	1.40	3.22b	1.32d
SEM		0.01	0.10	0.02	0.40	0.25	0.15	0.08	0.07
Day means	60	3.91	1.66	0.49	0.94	3.32	2.03	4.03	2.06
	180	3.95	2.49	0.36	4.55	5.05	1.45	3.28	1.42
Treatment	CON	3.93	1.85	0.42	2.20	3.88	2.33	3.69	1.80
	LB	3.94	2.09	0.40	2.42	3.70	1.73	3.75	1.87
	PAP	3.94	2.03	0.45	3.05	3.98	1.17	3.65	1.72
	LBPAP	3.92	2.32	0.45	3.33	5.17	1.74	3.54	1.57
P-value									
D		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.026	< 0.001	< 0.001
T		0.113	0.006	0.023	< 0.001	0.04	0.027	0.008	< 0.001
D×T		0.002	< 0.001	0.003	< 0.001	0.327	0.322	0.039	0.002

Mean values with different letters in each column differ significantly ($P<0.05$).

**Figure 1.** FTIR spectra of high-moisture corn silage.

Discussion

Silage fermentation involves complex material transformations and microbial activity. Compared to other materials such as oats and fescue, HMC has lower moisture content and microbial activity. In this study, the inoculation of *L. buchneri* and papain significantly enhanced the fermentation profile, likely due to proteinase-mediated protein degradation, which released amino acids and other nitrogenous compounds. This provided sufficient nitrogen and carbon sources for lactic acid bacteria, promoting the production of acidic substances and maintaining the acidic environment necessary for fermentation. AN served as a key marker of protein breakdown. After fermentation, extensive hydrolysis of zein and GLU disrupted the starch-protein matrix, improving the accessibility of starch particles to rumen microorganisms. FTIR analysis revealed no new absorption peaks before and after fermentation, indicating no formation of new functional groups. However, the decreased intensity of the amide I region over fermentation time reflected the disruption of the rigid protein structure and its transition into a more disordered state. The synergistic action of microbes and enzymes accelerated this process, enhancing fermentation efficiency and improving feed digestibility.

Conclusions

This study demonstrates that the synergistic fermentation of *L. buchneri* and papain can effectively improve the silage quality of HMC, while accelerating the disruption of the protein's ordered structure. This approach holds potential for enhancing feed utilization efficiency in ruminant animals.

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Are Whole-Crop Silages of Cereals and Cereal-Legume Intercrops a Good Alternative for Maize Silage?

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Keywords: whole-crop silage, fermentation, nutritional value

Introduction

The cultivation of silage maize is becoming more and more challenging over the years in several regions, due to extremere weather conditions (ranging from wet and cold periods to warm and dry periods), more stringent fertilization regulations, reduced availability of chemical herbicides, herbicide-resistance of weeds, etc. Whole-crop silage (WCS) of cereals in pure stand or intercropped with legumes are interesting candidate-alternatives for multiple reasons: 1) These crops are harvested earlier in the season than whole-crop maize, making them less prone to heat and/or drought stress during summer, 2) For intercrops with legumes, fertilization can be reduced thanks to the nitrogen fixing ability of legumes, 3) Cereals (whether or not intercropped with legumes) form a denser crop than maize, reducing weed development and thus also the need for herbicides, etc. To evaluate their potential, it is crucial to gain more insight in the silage fermentation characteristics and in the nutritional value for ruminants.

Materials and methods

In 2023, an ensiling trial with microsilos (volume of 2.75 liter; n=4) was performed with different winter crops (Table 1) harvested as WCS. At silo opening after 90 days, pooled samples per treatment were taken for determination of fermentation characteristics and nutritional value (cfr. Van den Bossche *et al.*, 2023).

Table 1. Treatments included in ensiling trial with whole-crop silage of cereals and cereal-legume intercrops.

Trt	Crop(s)	Cultivar(s)	Sowing density	Harvest date
1	barley	HB SY 220061 VGOLD585 ¹	180 seeds/m ²	June 15 th , 2023
2	barley	SU Hylona ²	180 seeds/m ²	June 15 th , 2023
3	barley-pea	HB SY 220061 VGOLD585 – Flokon ³	130-40 seeds/m ²	June 15 th , 2023
4	barley-pea	HB SY 220061 VGOLD585 - Flokon	100-50 seeds/m ²	June 15 th , 2023
5	triticale-pea	RGT Eleac ⁴ – Asteroid ³	130-50 seeds/m ²	June 21 st , 2023
6	triticale-pea	Lumaco ⁵ – Asteroid	130-50 seeds/m ²	June 21 st , 2023
7	triticale	RGT Eleac	350 seeds/m ²	July 4 th , 2023
8	triticale	Lumaco	350 seeds/m ²	July 4 th , 2023
9	triticale	Kasyno ⁶	350 seeds/m ²	July 4 th , 2023
10	triticale	Temuco ⁷	350 seeds/m ²	July 4 th , 2023
11	triticale-faba bean	RGT Eleac – Tundra ²	175-20 seeds/m ²	July 4 th , 2023
12	wheat-faba bean	Extase ⁴ – Tundra	130-20 seeds/m ²	July 4 th , 2023
13	méteil mixture	Proti Méteil ⁴	120 kg/ha	July 4 th , 2023
14	méteil mixture	own mixture ⁸	120 kg/ha	July 4 th , 2023

¹Syngenta, ²Limagrain, ³Agri-Obtentions, ⁴Jorion Philip Seeds, ⁵Arvesta, ⁶Danko, ⁷Lantmännen, ⁸Oats cv. Vodka⁴, rye cv. KWS Serafino⁵, triticale cv. Lumaco⁵ and pea cv. Flokon³ (resp. 75-50-50-45 seeds/m²).

Results

The fermentation characteristics and nutritional value parameters of the different WCS are presented resp. in Table 2 and Table 3. In line with large differences in dry matter (DM) content (range 301 - 555 g kg⁻¹ FM), the fermentation characteristics were highly variable between treatments. The pH values ranged from 4.21 to 5.12, showing a good acidification. However, ammonia fractions were in general quite high (range 8.6 - 19.8 %) and high values for butyric acid and alcohols (resp. range 0.2 - 26.4 and 3.0 - 37.3 g kg⁻¹ DM) were detected too, indicating a sub-optimal silage fermentation process (McDonald *et al.*, 1991). Also for the nutritional value parameters, large differences were found between treatments.

From a farmer's perspective, Net Energy for Lactation (NEL) and true protein digested in the small intestine (DVE; Tamminga *et al.*, 1994) are the most important parameters for ration calculation. Taking whole-crop maize silage with an average NEL value of 6.89 MJ kg⁻¹ DM and an average DVE content of 53 g kg⁻¹ DM (De Brabander, 2012; Eurofins, 2023) as a reference, the cereal and cereal-legume WCS perform less well. From the tested treatments, the highest NEL values were obtained for triticale in pure stand.

Table 2. Fermentation characteristics: pH, ammonia fraction (NH₃-N/total N), lactic acid (LA), acetic acid (AA), butyric acid (BA), alcohols (Alc.), sum of fermentation products (FP) and ratio of lactic acid to acetic acid (LA/AA).

Trt	Crop	DM	pH	NH ₃ -N/total N	LA	AA	BA	Alc	FP	LA/AA
		g kg ⁻¹ FM		Ratio			g kg ⁻¹ DM			Ratio
1	barley	433	4.44	8.76	29.8	6.18	4.58	28.9	69.4	4.82
2	barley	421	4.85	8.60	9.34	2.28	8.03	37.3	56.9	4.10
3	barley-pea	387	4.67	11.46	12.1	2.44	17.9	31.3	63.7	4.95
4	barley-pea	377	4.66	10.76	17.9	2.43	14.9	32.6	67.8	7.36
5	triticale-pea	315	4.35	15.31	34.5	5.77	26.4	10.5	77.1	5.97

6	triticale-pea	301	4.21	15.09	63.9	9.96	2.84	5.54	82.2	6.42
7	triticale	555	4.97	12.18	7.79	3.70	3.46	13.8	28.7	2.11
8	triticale	521	5.12	15.51	8.48	2.25	6.19	13.1	30.0	3.77
9	triticale	473	4.91	19.81	13.9	2.28	10.5	16.3	43.0	6.08
10	triticale	524	5.01	14.38	11.6	1.96	5.57	12.7	31.8	5.92
11	triticale-faba bean	468	5.02	15.95	8.14	2.23	9.57	16.4	36.3	3.65
12	wheat-faba bean	497	5.07	17.21	8.86	2.59	8.16	10.2	29.8	3.42
13	méteil mixture	423	4.43	11.06	40.1	8.16	0.17	3.02	51.5	4.92
14	méteil mixture	485	4.62	9.19	20.1	3.23	11.7	4.98	40.1	6.23

Table 3. Nutritional value parameters: crude protein (CP), starch, crude fiber (CF), fermentable organic matter (FOM), true protein digested in the small intestine (DVE), net energy for lactation (NEL), rumen-degradable protein balance (OEB), rumen-undegradable protein (RUP) and rumen-undegradable starch (RUS).

Trt	Crop	CP	Starch	CF	FOM	DVE	NEL	OEB	RUP	RUS
		g kg ⁻¹ DM				MJ kg ⁻¹ DM		----- % -----		
1	barley	85	380	224	498	44	5.44	-21	32.8	19.4
2	barley	79	275	243	517	42	5.51	-26	30.7	20.7
3	barley-pea	70	274	223	532	41	5.77	-32	28.4	19.6
4	barley-pea	84	235	253	517	43	5.44	-22	29.9	20.1
5	triticale-pea	113	110	325	498	42	4.83	6	25.6	21.3
6	triticale-pea	122	139	319	525	48	5.02	9	25.0	18.6
7	triticale	76	357	258	537	41	5.27	-28	27.3	12.0
8	triticale	72	298	277	514	36	5.01	-28	28.3	12.6
9	triticale	83	247	243	560	43	5.71	-22	19.1	31.5
10	triticale	85	248	256	574	46	5.65	-24	21.1	29.8
11	triticale-faba bean	80	277	254	539	40	5.41	-23	25.1	12.3
12	wheat-faba bean	72	277	268	527	37	5.21	-28	19.1	34.6
13	méteil mixture	142	130	323	503	55	4.81	16	31.8	26.1
14	méteil mixture	85	175	290	521	43	5.25	-23	27.5	32.9

Conclusions

From a nutritional point of view for ruminant feeding, whole-crop silages of cereals and cereal-legume intercrops can't live up to the high standards set by whole-crop maize silage. Still, there are possibilities towards optimization of the silage fermentation process (e.g. by silage additive application), which might have a beneficial effect on the nutritional value of cereals and cereal-legume intercrop silages.

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Effect of a Silage Inoculant on Fermentation Quality and Biogas Yield of Different Grass Silage Varieties

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Keywords: silage inoculant, biogas yield, grass silage

Introduction

Many biogas plants have been built in the recent decades, especially in Western Europe, but also in other continents such as Asia or North America. As a result, the need for substrates to feed the digester has increased (Schnittenhelm, 2008). The most competitive crop is corn silage but rising prices and environmental concerns are making other substrates more acceptable. Grassland is a low-cost biomass resource for biogas production in temperate regions. However, high variability in biogas yield (BY) can occur due to many factors (e.g. plant species, maturity, harvest time and fermentation quality) (DLG, 2015). In practice, farmers need to ensile large amounts of silage for use in a biogas plant and harvesting can take several days, resulting in large variations in dry matter (DM), water soluble carbohydrates (WSC), crude fiber (CF) or crude protein (CP). In addition, weather conditions during harvest and grass varieties vary among countries and farmers are looking for a silage inoculant that protects against anaerobic fermentation losses, increases aerobic stability and BY. Therefore, the aim of this study was to evaluate whether a silage inoculant based on homofermentative and heterofermentative lactic acid bacteria (LAB) improves fermentation, aerobic stability and BY of two different grass silage varieties.

Materials and methods

In 2023, two different grass silage varieties were ensiled in laboratory mini silos (1.5 L) in northern Germany. Variety 1: 1st cut Italian ryegrass (IR: DM: 28.9%; WSC: 24.0% DM; CP: 11.9% DM; CF: 18.4% DM); Variety 2: 2nd cut Tall fescue (TF: 31.6% DM; 13.4% WSC in DM; 13.7% CP in DM; 23.9% CF in DM). Two treatments were carried out with n= 3 replications:

1) CON: untreated control; 2) LAB: a mixture of homo - and heterofermentative LAB (*Silasil Energy.SG: Lentilactobacillus buchneri*, *Lentilactobacillus plantarum*, *Loigolactobacillus coryniformis*, *Enterococcus lactis*, application rate: 200,000 CFU/ g FM).

The mini silos were opened after 6, 14 and 90 days (d) of storage at 20°C. Measured parameters: pH value and fermentation pattern at day 6 and 14; DM, pH, fermentation pattern, fermentation weight losses- determined based on the mass difference of the mini silos before and after ensiling and expressed as percentage fresh matter, aerobic stability according to Honig (1990) and biogas yield (BY - measured in batch anaerobic fermenters at the temperature of 38°C according to German Standard VDI 4630) at day 90. For statistical evaluation the medians were calculated and the non-parametric Mann Whitney U-Test was performed on the parameters to test for significant differences ($P < 0.05$) between the control and the treated silages.

Results

For the grass variety IR, pH values of LAB treated silages were significantly lower than those of CON at all opening days (OD) - see table 1. In addition, lactic acid was increased with the use of LAB at all ODs, whereas no difference was observed for acetic acid concentration at all ODs. Furthermore, ethanol was increased at OD14 and OD90 for CON - probably due to activity of undesirable yeasts and/ or ethanol producing bacteria. Despite similar acetic acid concentrations, aerobic stability was higher with using the LAB mixture (10 d vs. 8.6 d). BY was significantly improved with using the LAB-mixture - this may be due to the improved and more efficient fermentation process.

For the grass variety TF, pH values of the LAB treated silage were significantly lower and lactic acid concentration was higher than that of the CON treated silages at all opening days ODs (table 1). This could be related to a strong activity of the homofermentative bacteria *L. plantarum*, *L. coryniformis* and *E. lactis* in the LAB-mixture, which also prevented the formation of butyric acid (LAB: 0.0% vs. CON: 0.35% DM). In contrast to the IR variety, acetic acid was significantly improved at OD14 and OD90 - this is due to the activity of *L. buchneri*. Therefore, aerobic stability at OD90 was improved by using the LAB-mixture (10 d vs. 6.3 d). Furthermore, BY was significantly improved with 578 Nl/ kg DM observed for CON and 584 Nl/ kg DM for the LAB-mixture. In general, BY was higher for the IR grass in contrast to the TF grass, this can be attributed to the higher WSC and lower CF content and thus better digestibility of the IR variant.

Table 1 Silage parameters of grass silage variety Italian ryegrass (IR) and Tall fescue (TF) at different opening days (OD) for control (CON) and the treated LAB-mixture (LAB)

Parameter IR grass silage	OD6		OD14		OD90	
	CON	LAB	CON	LAB	CON	LAB
DM _c (%)	28.7	28.6	28.4	28.7	26.6 ^a	27.5 ^b
LA (% DM)	6.12 ^a	8.86 ^b	7.20 ^a	10.05 ^b	9.87 ^a	14.18 ^b
AA (% DM)	0.80	0.83	0.93	1.14	3.05	3.24
BA (% DM)	0.00	0.00	0.00	0.00	0.00	0.00
Eth (% DM)	0.45	0.35	0.95 ^a	0.66 ^b	0.86 ^a	0.44 ^b
pH	4.57 ^a	4.20 ^b	4.29 ^a	3.94 ^b	4.13 ^a	3.84 ^b
WL (%)					1.56 ^a	0.97 ^b
AS (days)	-	-	-	-	8.6 ^a	10.0 ^b
BY (NI/ kg DM)	-	-	-	-	621 ^a	637 ^b

Parameter TF grass silage	6		14		90	
	CON	LAB	CON	LAB	CON	LAB
DM _c (%)	31.9	31.8	31.6	31.5	31.8 ^a	30.4 ^b
LA (% DM)	3.07 ^a	7.61 ^b	5.27 ^a	10.43 ^b	5.66 ^a	9.97 ^b
AA (% DM)	0.89	0.84	0.87 ^a	1.25 ^b	0.94 ^a	3.62 ^b
BA (% DM)	0.00	0.00	0.00	0.00	0.35 ^a	0.00 ^b
Eth (% DM)	0.81 ^a	0.35 ^b	0.74 ^a	0.41 ^b	1.31 ^a	0.64 ^b
pH	5.08 ^a	4.13 ^b	4.70 ^a	3.91 ^b	4.53 ^a	3.86 ^b
WL (%)	-	-	-	-	2.23 ^a	1.10 ^b
AS (days)	-	-	-	-	6.3 ^a	10.0 ^b
BY (NI/ kg DM)	-	-	-	-	578 ^a	584 ^b

DM_c = DM corrected, LA=Lactic acid; AA= Acetic acid; BA= Butyric acid; Eth= Ethanol; WL= Weight losses in % FM; AS= Aerobic stability (max. length: 10 days); BY= Biogas yield; ^{a,b} symbolize significant differences ($P < 0.05$)

Conclusion

The present study demonstrates the beneficial effects of a LAB-mixture containing homo- and heterofermentative bacteria on fermentation success, aerobic stability and biogas yield of different grass silage varieties. Thus, the tested LAB-mixture may help farmers who are looking for a solution to improve grass silages with different quality due to variety, DM and crude nutrients to be fed to biogas plants.

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Inhibition of Clostridia Growth by *Lactiplantibacillus plantarum* and *Lactococcus lactis*

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Keywords: *Lactiplantibacillus plantarum*, *Lactococcus lactis*, Clostridia inhibition, silage

Introduction

Clostridia are undesired microorganisms in silage. Anaerobe spoilage of low dry matter silage is often caused by unrestricted clostridia growth. For feeding purposes it is very important to keep counts of clostridia spores at a low level to avoid health problems and reduction in animal performance.

In the study presented, a broad range of lactic acid bacteria were screened for their ability to inhibit clostridia growth. Two new isolated strains, *Lactiplantibacillus plantarum* (LP) DSM 34271 and *Lactococcus lactis* (LC) DSM 34262, showed a good effect against clostridia in agar diffusion tests.

Based on these results, the two newly isolated lactic acid bacteria were inoculated in three different, slightly wilted grass materials to investigate their effect on quality parameters in comparison to an untreated control in the ensiling process.

Materials and Methods

Agar Diffusion Test for Studying Clostridia Inhibition

Clostridium tyrobutyricum DSM 2637 spores have been diluted to reach semi-confluent growth and enclosed into RCM agar plates. Supernatants of more than 200 strain cultures (80 µl) were put into wells and incubated anaerobically (AN) at 37°C for 24 h. A clear zone (halo) around the well shows the inhibition of clostridia [1].

Ensiling Material and Treatment

Grass material from three sources with dry matter concentration in the range of 27 – 36% were slightly wilted. Each of the chopped grass materials was ensiled once as untreated control and once treated with *Lactiplantibacillus plantarum* DSM 34271 or *Lactococcus lactis* DSM 34262. The lactic acid bacteria were sprayed onto the grass as a liquid bacterial solution with a concentration of 2.5×10^5 cfu/g grass. Laboratory silos (6-L) were analyzed after 90 days of storage at 20°C.

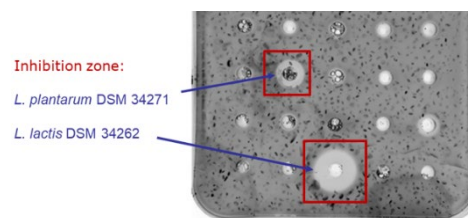
Measured Parameters

The fermentation profile was measured from an aqueous silage extract (50 g silage extracted with 250 g distilled water) using HPLC (Agilent 1100, Column: Concise Separations IC Sep ICE-ION 300). Ammonia-N was determined by an ammonia sensitive electrode.

Agar Diffusion Test for Studying Clostridia Inhibition:

Table 1 / Figure 1: Inhibition zones of investigated lactic acid bacteria.

Strain	Incubation [C°]	Halo [mm]
<i>L. plantarum</i> DSM 34271	37°C / AN	1.5
<i>L. lactis</i> DSM 34262	37°C / AN	5.0



The two selected lactic acid bacteria showed a clear inhibition zone (Table 1, Figure 1). *Lactococcus lactis* DSM 34262 had a better effect against the test strain *C. tyrobutyricum*, compared to *L. plantarum* DSM 34271.

Table 2: Comparison of the parameters of mean values from three silage trials using slightly wilted grass material after 90 days of storage.

Parameter	Unit	Mean Values of three Silage Trials / 90 d				
		Control	LP (DSM 34271)	p-value	LC (DSM 34262)	p-value
DM	g/100 g FM	31.0	32.1	0.159	31.5	0.451
pH – value		4.72	3.98*	<0.001	4.25*	0.013
Ammonia-N	g/100 g DM	0.30	0.15	0.064	0.22	0.192
Weight losses	g/100 g FM	2.88	1.36*	0.004	1.84*	0.019
Lactic acid	g/100 g DM	3.11	7.65*	<0.001	5.98*	<0.001
Acetic acid	g/100 g DM	1.33	0.68	0.083	0.93	0.268
Butyric acid	g/100 g DM	0.56	0.05*	0.027	0.20	0.130

* Indicates mean values within a line are significantly different at $p < 0.05$ (Mann-Whitney-Test) in comparison to the control group.

The dry matter of the grass ensiled ranged from 27% to 36%. The mean pH-value in the untreated control was 4.72. Inoculated silages reached significantly lower pH values of 3.98 and 4.25, respectively. This indicates improved preservation in the treated grass. Corresponding to pH pattern, treated silage showed significantly higher concentration of lactic acid of 7.65 and 5.98 g, compared to controls' 3.11 g per 100 g DM, respectively. Treated materials' lower concentrations of butyric acid and ammonia-N are consistent with pH and lactic acid pattern. Additionally, silage fermenting to higher lactic acid concentrations lost less weight, supposed by inhibiting spoilage microorganism's growth (Control: 2.88 g/100 g FM; Treatment: 1.36 and 1.84 g/100 g FM). Values are shown in Table 2.

Conclusions

In a screening project two lactic acid bacteria strains inhibited *Clostridia* significantly. Figure 1 shows the result of an agar diffusion test. The two isolates, *Lactiplantibacillus plantarum* DSM 34271 and *Lactococcus lactis* DSM 34262, inhibit clearly the growth of the test strain *C. tyrobutyricum* DSM 2637.

Based on these results, we conducted silage trials. The two selected lactic acid bacteria were tested in slightly wilted grass with high risk for clostridia contamination. The results showed that the two strains were able to improve silage quality in the three independently conducted ensiling experiments (Table 2). The main effects were a significant decrease of pH with corresponding increased production of lactic acid. Additionally, a reduced activity of clostridia can be assumed to be indicated by lower amount of butyric acid formed.

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Fertilizer N Effects on Silage Quality of Grass-legume and Herb Mixtures

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Keywords: climate change, mix cultivation, mix silage, laboratory jar silo, lactic acid bacteria

Introduction

Climate change adversely impacts grassland yield, stability and feed availability, posing challenges for sustainable animal nutrition. Plants grown in mixtures respond less strongly to drought stress (Finn et al. 2018). Herbal mixtures not only have good forage qualities but are also suitable for preservation as silage (Moloney et al. 2020), if the requirements for rapid wilting and/or effective preservative application are met. This study aimed to evaluate the silage quality of herbal mixtures grown under different nitrogen fertilization levels and assess the effects of a biological silage additive (SA) containing homo- and heterofermentative lactobacteria.

Materials and Methods

A plot trial (2020–2022) at the Schuby trial site in Northern Germany was established to cultivate four forage types (n=4) under nitrogen applications of 40 (N1) and 220 kg N/ha/year (N2). Forage types were perennial ryegrass (*Lolium perenne*), a ryegrass-white clover (*Trifolium repens*) mixture (Mix 1) and a herb mixture of ryegrass, white clover, ribwort plantain (*Plantago lanceolata*) and chicory (*Cichorium intybus*) sown as mixed and strip variants (Mix 2).

The third harvest (2022) was selected for ensiling trial, yield fractions are mentioned in Table 1. The material wilted up to 35 % dry matter (DM) was chopped to 1–2 cm. Three jar silos with a volume of 0.5 L respectively 1.7 L were filled with forage and compacted to a target density of 190 kg DM/m³. Control variant (CON) was not inoculated. Treated silages were inoculated with a commercial SA containing *Lactobacillus plantarum* LSI NCIMB30083/1k20736, *Pediococcus pentosaceus* P6 DSM23688/1k1010, *Lactobacillus buchneri* DSM22501/k20738 and *Lactobacillus kefir* DSM19455/1k20742 at a rate of 300,000 CFU/g. The airtight sealed silos were weighed and stored at 25 °C. After 3 days, the smaller jars were opened to measure the pH. After 90 days, the 1.7 L jars were weighed again to determine ensiling losses and silage samples were taken for pH measurements, external laboratory analyses of feed, fermentation and hygienic quality as well as for aerobic stability testing. Aerobic stability was defined as the time until silage temperature exceeded ambient temperature by 3 °C. Statistical analysis was conducted in SPSS® 2023 using the Kruskal-Wallis test.

Table 1. Mean values of dry matter yield and fractional composition of various plant species in the 3rd cut 2022 of the plot trial (n=4).

Fertilization level		Mix 1		Mix 2	
		N1 ¹	N2 ²	N1	N2
DM yield dt/ha		10.1	11.05	9.3	10.15
Fraction [%]	Perennial ryegrass (<i>Lolium perenne</i>)	49.1	84.3	39.92	71.81
	White clover (<i>Trifolium repens</i>)	42.6	15.7	48.18	8.82
	Ribwort plantain (<i>Plantago lanceolata</i>)	-	-	5.72	12.62
	Chicory (<i>Cichorium intybus</i>)	-	-	5.40	3.86
	diverse, non-sown species	8.30	0.00	4.18	2.89

¹N1= 40 kg N/ha/year, ²N2= 220 kg N/ha/year

Results and discussion

Table 2 shows the quality parameters of the silage mixtures. Crude protein (CP) was higher in mixes with a lower fertilization level due to the higher proportion of white clover in the starting material (Table 1). Crude ash was low in all cases. Crude fiber content was affected neither by mixture nor by N-fertilization. However, both mixes with higher fertilization level demonstrate better digestibility than the N1 mixes, evident from fiber fractions, sugar and energy content caused by the higher proportion of perennial ryegrass. All silages showed a strong acidification, a minor DM loss and lacked butyric acid, propionic acid, 1,2-propanediol and molds (Data not shown), indicating optimal silage fermentation.

In N1-fertilized mixes, lactic acid (LA) content was significantly higher than in N2-fertilized mixtures, with the differences being more pronounced in Mix 2. This finding was unexpected, as the fresh material had a lower sugar content, a higher buffering capacity and similar pH values after 90 days of fermentation. Similarly, Moloney et al (2020) reported higher LA concentrations in unfertilized multi species mixtures. The application of SA further increased LA concentrations, resulting in a significantly faster and more pronounced reduction in pH. In contrast, acetic acid (AA) content was significantly higher in N2-fertilized mixtures. At both fertilization levels, control treatments exhibited significantly higher AA content, potentially due to slower fermentation processes or enhanced activity of natural heterofermentative bacteria. Ethanol content remained below 1 % across all mixtures. The low ammonia levels observed indicated minimal protein degradation.

Except for the SA-treated silage from the lower fertilized Mix 1, no reheating was observed in the silages during aerobic storage. However, during the visual assessment following the aerobic stability test, some silages that remained cold still exhibited signs of aerobic spoilage.

Table 2. Mean values (n=3) of silage quality parameters of grass-legume mixtures (Mix 1) containing additional herbs (Mix 2), cultivated under different N fertilizer levels, after 90 days of fermentation with (SA) or without inoculation (CON). Means within a line not followed by the same letter are significantly different ($P \leq 0.05$).

Parameters ⁴		MIX 1				MIX 2				SEM ³
		N1 ¹		N2 ²		N1		N2		
		CON	SA	CON	SA	CON	SA	CON	SA	
DM	[%]	38.0 ^a	35.6 ^e	38.0 ^a	38.5 ^{ab}	36.5 ^c	38.0 ^d	37.8 ^a	38.8 ^b	0.21
CA		9.7 ^a	9.0 ^b	8.2 ^c	7.9 ^c	9.4 ^a	8.7 ^b	7.7 ^c	8.2 ^c	0.14
CP		15.5 ^a	15.8 ^a	13.8 ^b	13.5 ^b	15.6 ^{ac}	15.6 ^c	12.9 ^b	13.9 ^b	0.25
CF		23.0 ^a	22.7 ^a	23.7 ^c	23.0 ^{ac}	22.4 ^b	22.6 ^{ab}	23.2 ^{bc}	23.2 ^c	0.10
aNDFom	[% DM]	38.4 ^a	39.2 ^a	44.4 ^c	43.7 ^c	36.6 ^b	37.2 ^b	43.4 ^c	42.9 ^c	0.64
ADFom		28.0 ^a	27.9 ^a	25.6 ^b	25.0 ^c	27.6 ^a	28.0 ^a	24.7 ^b	25.2 ^{bc}	0.30
Sugar		5.8 ^a	5.7 ^a	10.9 ^c	10.5 ^c	5.6 ^a	4.2 ^b	10.6 ^c	8.8 ^d	0.53
NEL	[MJ/kg DM]	6.2 ^a	6.1 ^a	6.6 ^c	6.5 ^d	6.1 ^b	6.0 ^b	6.5 ^{bc}	6.5 ^{cd}	0.04
NH ₃ -N	[% N _{total}]	5.9 ^a	4.7 ^{bc}	5.0 ^c	4.6 ^c	5.2 ^{ad}	5.3 ^a	3.8 ^d	4.9 ^c	0.14
LA		5.8 ^a	8.4 ^b	5.4 ^e	7.5 ^f	6.0 ^c	7.7 ^d	4.0 ^g	6.0 ^h	0.28
AA	[% DM]	1.9 ^a	0.8 ^b	1.6 ^e	1.1 ^f	1.4 ^c	1.1 ^d	1.6 ^{eg}	1.5 ^h	0.06
Ethanol		0.5 ^a	0.7 ^{ad}	0.5 ^a	0.9 ^d	0.6 ^b	0.5 ^a	0.5 ^a	0.4 ^c	0.04
Yeasts	CFU log ₁₀ /g	1.0 [*]	4.1	1.3 [*]	5.4	0.0	1.3 [*]	4.5	2.3	-
DM loss	[%]	4.4 ^a	4.3 ^a	4.4 ^a	4.4 ^{ac}	4.2 ^b	4.0 ^{ab}	4.6 ^a	4.3 ^c	0.07
pH 3 d		5.1 ^a	4.2 ^b	4.5 ^c	4.3 ^e	4.6 ^c	4.4 ^d	4.5 ^c	4.5 ^c	0.05
pH 90 d		4.2 ^a	3.9 ^b	4.1 ^a	3.9 ^b	4.1 ^a	4.0 ^d	4.2 ^c	4.0 ^d	0.02

¹N1= 40 kg N/ha/year, ²N2= 220 kg N/ha/year, ³SEM= Standard error of mean.

⁴Parameters: CA=Crude Ash, CP=Crude Protein, CF=Crude Fiber, NEL=Net Energy for Lactation, LA=Lactic Acid, AA=Acetic Acid,

*Only in one sample yeasts were detected.

Conclusion

The nutritional value of the two mixtures, with or without the inclusion of herbs, was predominantly influenced by nitrogen fertilization levels. Lower fertilization levels resulted in an increased proportion of white clover, thereby enhancing the crude protein content of the silage. In contrast, higher fertilization promoted a greater proportion of herbs and, more notably, a dominance of perennial ryegrass, leading to higher energy contents. The successfully wilted mixtures exhibited a rapid and substantial reduction in pH, driven by efficient lactic acid production, which was further enhanced by the application of a biological silage additive.

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Comparison of Estimated Biogas Yield with Regression Formula and Measured Biogas Yield with Batch Test of Different Grass Silages

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Keywords: grass silage, biogas yield, methane yield

Introduction

The growing amount of biogas plants in Western Europe in the last decades increases the need for substrates as feedstock for the digester (Schnittenhelm, 2008). So far corn is the most competitive crop, but the increasing prices and harmful effects on the environment, make other substrate more compatible. Grassland represents a low-cost biomass resource for biogas production in temperate regions. However, a high variation of biogas (BY) and methane yield (MY) can occur due to many factors (e.g. plant species, maturity, harvest time) (DLG, 2015). In practice it is important to assess the quality of the substrate for management reasons, e.g. to determine the feeding rate. Experimental batch tests according to VDI 4630 are time consuming and expensive. Therefore, several authors have tried to establish a relationship between the chemical composition of substrates and their BY/MY. Hence, in this study, batch test results were compared with the long-established Weissbach formula (Weissbach, 2008) to investigate potential of grass silages as an alternative biogas substrate.

Materials and methods

The forages tested in this study originate from 13 different grass silages collected from farms in UK or Germany. They were harvested as 1st or 2nd cut and ensiled for at least more than twelve weeks. Forage samples were analyzed for dry matter (DM), crude protein (CP), crude fiber (CF), hemicellulose (HC), sugar, crude fat (CL) and ash. The average composition of the silages is shown in Table 1. According to Weissbach (2008), the fermentable organic dry matter (FoTS) was determined with the CF and ash content and the theoretical biogas and methane yield (BY_W and MY_W) were calculated for each silage.

Table 11. Overview of the average chemical composition of the 13 grass silages included in this study.

	Dry matter %	Crude protein % DM	Crude fat % DM	Sugar % DM	Crude fiber % DM	Hemicellulose % DM	Ash % DM	ME MJ/kg DM
Mean	28.7	14.8	3.8	7.3	24.9	21.1	8.6	10.6
SD	3.6	3.2	0.4	4.7	5.7	3.6	2.0	1.0
Min	23.5	7.1	2.6	1.5	17.6	16.6	4.0	8.5
Max	36.7	17.7	4.5	14.0	35.9	27.3	10.8	11.9

In addition, the experimental BY_B and MY_B (biogas yield and methane yield generated from batch test) of all silages were measured in batch anaerobic fermenters according to the German standard VDI 4630. Statistical evaluation was performed using R studio (Posit team, 2024). First, Pearson's correlation coefficients were determined between the experimental BY_B/MY_B and the chemical compositions and second, two linear models including the fixed effects CP, CL, and CF were used to evaluate the effect on the BY_B or MY_B. The analysis was carried out at a significance level of 5%.

Result

The average BY_B and MY_B (NL/kg DM) of all 13 silages was higher with 606.6 (± 38.9) and 348.5 (± 29.4) analyzed with the batch test compared to the theoretical values BY_W and MY_W according to Weissbach (2008) with 555.6 (± 54.6) and 291.7 (± 28.7); this corresponds to an average percentage deviation of 17.7% and 24.8% for the theoretical BY_W and MY_W compared to the experimental batch values (BY_B/MY_B). Figure 1 shows the individual experimental and theoretical BY and MY for each silage. However, these determined BY/MY are thus within the range of the reference values specified by the DLG, while corn producing on average 20% more MY (DLG, 2015).

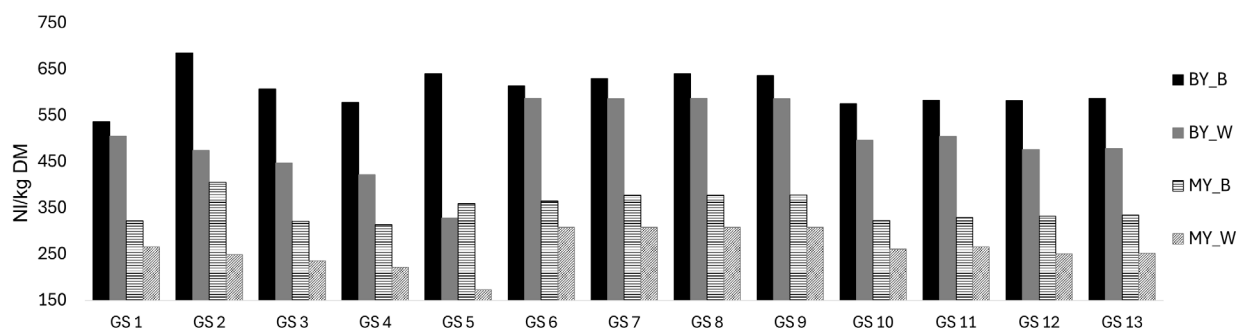


Figure 12. Overview of the experimental measured biogas and methane yield (BY_B; MY_B) and the theoretical values (BY_W; MY_W) according to Weissbach (2008) of each silage.

For CL, a moderate positive correlation with BY_B was calculated; for MY_B, a moderate positive correlation was also obtained for CL and negative for CF and HC, see Table 2. However, Dandikas et al. (2015) obtained a higher correlation coefficient for HC (BY = 0.63/MY = 0.59) and a moderate value for CP, but a weak correlation with CF and CL. They argued that the Weender analysis does not describe the cell wall specifically enough to use the parameters for the prediction of the BY/MY. They suggested using the Van Soest analysis (NDF/ADF/ADL) and found a high correlation between the ADL content and the BY/MY, but only 50% ($R^2 = 0.53$) of the variance in BY could be explained by ADL. They concluded that a monocausal regression was not suitable for a prediction and created a multiple linear regression model, which included CP, HC and ADL as regressors to obtain the best prediction of the BY. The linear models in this study showed similar results, with a significant influence on the BY_B and MY_B found for CP, CF and CL, see Table 2. Dandikas et al. (2015) reflected that they did not find a significant influence of the CL content, due to the fact that the CL variations were low in the samples of different growth stages and species in their study. The effects of the below mentioned models reflect the need to describe the actual physiological stage of the plant and not only its composition. So, the protein content decreases during the plant senescence, while the fiber and ADL content increases, attributing to a lower digestibility, resulting in lower BY/MY.

Table 2. Person's correlation coefficients (COR - left column) between the experimental measured biogas yield (BY_B) as well as the measured methane yield (MY_B) and the chemical components of the grass silages and P-values (LM -right column) of the linear models with the dependent variable BY_B or MY_B and the chemical component parameters.

	Crude protein		Crude fiber		Crude fat		Ash		Hemicellulose	
	COR	LM	COR	LM	COR	LM	COR	LM	COR	LM
BY_B	-0.03	0.005	-0.14	0.040	0.35	0.003	0.00	ns	-0.23	ns
MY_B	0.14	0.020	-0.38	0.010	0.39	0.010	-0.22	ns	-0.33	ns

ns = not significant

Conclusions

Based on the results of this study and considering the results of other studies, grassland samples with high protein and low fiber content (young material) are preferred feedstock for a biogas plant and could be an interesting alternative for corn. For a more realistic prediction of the biogas/methane yield a multiple regression model with a more detailed cell wall description is recommended.

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Effect of Different Silage Inoculants on Digestibility Parameters and Metabolizable Energy *In vivo* – A Meta-analysis

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Keywords: *in vivo* digestibility, silage inoculants, silage quality, sustainability

Introduction

Livestock production has a significant share in global greenhouse gas (GHG) emissions, with cattle representing the largest contributor to the sector emissions, driven by emissions from enteric fermentation (Gerber et al, 2013). Strategies in ruminant nutrition to reduce the emission intensity (g CO₂eq/kg energy corrected milk, or average daily gain in beef cattle) include improving forage quality and digestibility (Hristov, 2013). Silage inoculants based on lactic acid bacteria (LAB) are used to optimize the overall conservation process and thus silage quality, and their beneficial effects on digestibility and animal performance have been recognized (Kung et al, 1993; Kung et al, 2003). The objective of this study was to compare silages ensiled with different mixtures of LAB with corresponding untreated controls. The digestibility results and thereon derived energy values included in this meta-analysis were compiled from several *in vivo* digestibility studies in sheep.

Materials and methods

This meta-analysis bases on 14 *in vivo* digestibility studies, where the effect of three different silage inoculants (INO) on digestibility was tested against a non-treated control:

- 1) INO1: Mixture of homofermentative lactic acid bacteria (*L. rhamnosus* DSM 7133 and *E. lactis* DSM 7134, application rate: 100,000 CFU/g FM)
- 2) INO2: Mixture of homo- and heterofermentative lactic acid bacteria (*L. rhamnosus* DSM 7133, *L. plantarum* DSM 12836, *P. pentosaceus* DSM 12834, *L. buchneri* DSM 12856 and *L. brevis* DSM 12835, application rate: 100,000 CFU/g FM)
- 3) INO3: Mixture of homo- and heterofermentative lactic acid bacteria (*L. buchneri* DSM 12856, *L. plantarum* DSM 12837 and *P. pentosaceus* DSM 12834, application rate: 250,000 CFU/g FM)

The studies were performed between 1996 and 2002 at different institutes in Germany (Martin Luther University Halle-Wittenberg; University Stuttgart-Hohenheim; University Berlin; University München-Weihenstephan; Haus Riswick; LAZBW Aulendorf) with three different types of silages (A: alfalfa, G: grass, M: maize), differing in number of cuts, DM and maturity defined as crude fiber (CF), see Table 1.

Table 1. Overview of the used silages regarding dry matter (DM), crude fiber (CF) range and silage inoculant (INO1/2/3)

	N studies	DM range (%)	CF range (% DM)	Silage inoculant
Grass 1 st cut	6	22-50	21.7-27.4	INO1; INO2
Grass 2 nd cut	1	51	27.7	INO2
Alfalfa 1 st cut	2	35-56	26.6-27.2	INO1
Alfalfa 2 nd cut	1	40	28.0	INO2
Maize	4	25-40	17.7-25.6	INO1; INO2; INO3

The silages were ensiled for 90 days and the digestibility parameters and thereon derived metabolizable Energy (ME) were determined according to the guidelines of the German Society of Nutritional Physiology (GfE 1991; GfE 2001). Briefly: 10 to 21 days treatment diet adaption period followed by 7 to 10 days treatment diet trial period and fecal collection to determine the digestibility of the nutrients, 4 or 5 sheep per treatment.

Statistical evaluation was performed using R studio (Posit team, 2024). A linear mixed model including the random effect study and the fixed effect silage type (A, G, M) was used to evaluate the effect of each silage inoculant (INO1; INO2; INO3) on the ME estimation in comparison to the corresponding non-treated control. The analysis was carried out at a significance level of 5%.

Results

The model of the meta-analysis showed significantly higher estimated marginal means of the ME (MJ/kg DM) for the inoculated silages compared to the corresponding untreated control (INO1: 10.59 vs. 10.37; INO2: 10.20 vs. 9.77; INO3: 11.57 vs. 11.18), see Figure 1. The beneficial effect of LAB silage inoculants as presented here, has been observed as well by Aksu et al. (2004) determining improved fermentation and *in vivo* digestibility of DM and NDF from inoculated corn silage. The results confirmed the assumptions of other authors, attributing increased milk yield when feeding LAB treated silages to an increased ME and DM intake (Kung et al., 1993) or improved DM digestibility (Martinsson, 1992).

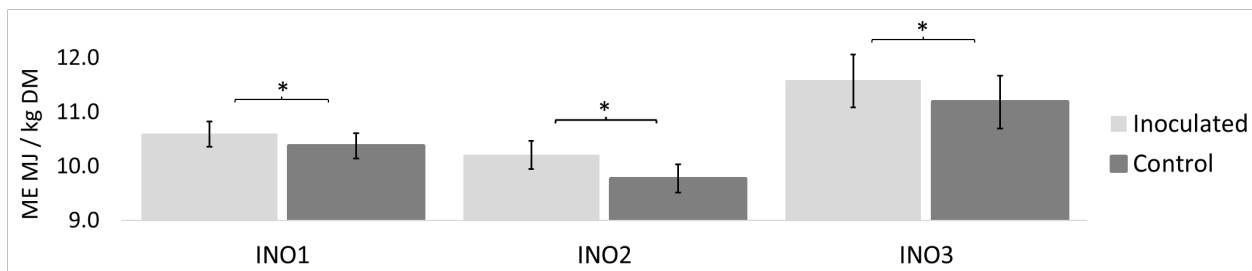


Figure 13: Estimated marginal means and standard error of the metabolizable energy (ME) of the three silage inoculants (INO1, used in alfalfa (A), grass (G), maize (M); INO2, used in A,G,M; INO3, used in M) and their controls ($P < 0.05$).

Wang et al. (2018), using different LAB strains of the same species but obtaining different results, emphasized the importance of the individual strains, which need to be evaluated for their potential effects as silage inoculant. Huyen et al. (2020) also noted a great potential of inoculants in terms of increasing digestibility and reducing emissions. These inoculant-related effects on silage quality and digestibility probably become more evident using the revised GfE feed energy evaluation system, considering the digestibility of organic matter and resulting in a higher ME prediction, especially for higher digestible forages (GfE, 2023). The current findings based on historical data support more recent studies on the impact of silage inoculants on silage conservation success and on the digestibility of the material, hence have the potential to be part of emission mitigation strategies in ruminant feeding.

Conclusions

A total of 14 different studies with grass, alfalfa and maize silage formed the data basis for the current meta-analysis. The study is confirming the wide range of the three tested silage inoculants used in different environments and improved the digestibility and metabolizable energy, which in turn provides potential to support a more efficient and sustainable milk production.

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Meta-analysis: DM loss Reduction and Improvement in Aerobic Stability Across Multiple Crops, DM Concentrations and Duration of Fermentation

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Keywords: silage, inoculant, multiple crops, fermentation time, DM loss, aerobic stability

Introduction

Silage inoculants can positively influence the fermentation process and the stability at feed out (Muck *et al.*, 2018). Silage inoculants are often developed for specific forage types, or even dry matter ranges within forage type. The increasing size of farm operations dictates that harvest will start when the forage dry matter is below optimum, continue when optimum dry matter conditions are met and finish well beyond optimum DM concentrations of the crops. To the dairy producer, limitations in the flexibility of a silage inoculant across DM concentrations may lead to increased complexity in switching from one type of inoculant to another as the harvest moves from wet to optimum to dry conditions. For some, the impact of complexity may result in not applying a silage inoculant – leading to increased dry matter losses and a resulting poorer economical return. The objective of this meta-analysis was to compile the results of trials conducted during the past 10 years using a combination of *Lactococcus lactis* and *Lentilactobacillus buchneri*, focusing on DM loss and aerobic stability of grass, grass/legume, alfalfa, and corn silages over a variety of DM concentrations and anaerobic fermentation times.

Materials and methods

The meta-analysis used trials (in total 19 trials with 30 contrasts) conducted in Europe (Denmark, Germany, Italy, Lithuania) on grass, grass/legume, alfalfa, and corn forages harvested at different DM (fresh or wilted) and stored as *either* mini silos (~1-10 kg) under controlled conditions *or* outdoor as big bales (350-850 kg) during fermentation for short (< ~2 weeks), medium (~1 month) or long (2-4 months) time. The following crops were chopped to a length of 2–3 cm before ensiling, prepared and ensiled in 3-20 liter mini-silos (or big bales) as follows: grass DM 20.8-56.3%, grass/legume DM 32.8-35.9%, alfalfa DM 32.0-34.1%, and whole-crop corn 32.6-38.9% with a forage chopper to a length of 1-2 cm. For the mini silo trials, 3-5 mini-silos (3-5 for fermentation quality and 3-5 for aerobic stability testing) were prepared. For bale trials, 5 bales per treatment (5 for fermentation quality and 5 for aerobic stability testing) were prepared. The trials included two treatments for each forage type and duration of anaerobic fermentation (AF): 1) control (C), with no inoculant applied, or 2) microbial-based inoculant (FC; *Lactococcus lactis* (DSM11037) and *Lentilactobacillus buchneri* (DSM22501); SILOSOLVE® FC, Novonesis, Lyngby, Denmark) applied at 150,000 CFU/g of fresh matter. AF time lasted from 2-120 days. Mini-silo silages were stored at room temperature ($20.0 \pm 1-1.6$ °C), while bales were stored in outdoor conditions. At termination of AF, samples of the ensiled crops (3-5 replicates for each treatment) were analyzed for DM. DM content was corrected for the loss of volatile compounds during oven drying (Weissbach and Strubelt, 2008) and DM loss was calculated. The silages were subsequently exposed to air by transferring them into open polystyrene boxes and storing them under controlled ambient conditions ($20.0 \pm 1-1.6$ °C), while bales were unwrapped of plastic and exposed to air under outdoor conditions. Temperature tracking (via probes or data loggers) was used as indication of aerobic stability. The aerobic challenge lasted 7-30 days. Aerobic stability was defined as the duration (in hours) for which the silage remained stable, i.e., before the temperature rose 2-3°C or more above ambient temperature. Each mini-silo or bale served as the experimental unit for statistical analysis, which was conducted using SAS software. The model included treatment as the main fixed effect, with statistical significance defined at $P \leq 0.05$.

Results

Across all four crops and duration of AF, the inoculation with *Lactococcus lactis* and *Lentilactobacillus buchneri* led to consistent reduction in DM loss (5.3% (C) vs. 4.0% (FC)) and improvement in aerobic stability (185 h (C) vs. 331 h (FC)). Details of the individual crops are shown in Table 1.

Table 1. Dry matter loss and aerobic stability (mean $\pm$ std dev) of grass, grass/legume, alfalfa, and corn silages at average 46.7 days of anaerobic fermentation (AF) ranging from 2-120 days.

	Grass ¹		% ^a	Grass/Legume ²		% ^a	Alfalfa ³		% ^a	Corn ⁴		% ^a
	C	FC		C	FC		C	FC		C	FC	
Dry matter (%)	33.0 (20.8-56.3)			35.2 (32.8-35.9)			33.5 (32.0-34.1)			35.9 (32.6-38.9)		
Fermentation DM losses (%)	4.7 $\pm$ 2.33	3.8 $\pm$ 2.31	61	8.7 $\pm$ 2.18	4.6 $\pm$ 1.13	83	5.5 $\pm$ 3.29	3.1 $\pm$ 1.58	83	5.0 $\pm$ 2.67	4.1 $\pm$ 1.97	63
Aerobic stability (hours)	163 $\pm$ 156.2	313 $\pm$ 197.8	100	157 $\pm$ 121.6	345 $\pm$ 280.2	83	321 $\pm$ 165.2	422 $\pm$ 200.4	100	174 $\pm$ 129.2	320 $\pm$ 219.8	93

C – non-inoculated; FC – inoculated with *Lactococcus lactis* and *Lentilactobacillus buchneri*. ^a% of trials with significant improvements on traits by applying FC (P < 0.05). ¹5 grass trials (average 40.1 days AF); ²3 grass/legume trials (average 77.7 days AF); ³3 alfalfa trials (average 64.3 days AF); ⁴8 corn trials (average (41.0 days AF)).

Across all four crops, the inoculation with *Lactococcus lactis* and *Lentilactobacillus buchneri* led to an increasing reduction in DM loss with increased duration of AF (3.0% vs. 2.9%; 5.8% vs. 4.5%; and 8.4% vs. 4.6% for (C) and (FC) at short, medium and long AF, respectively). In addition, an improvement in aerobic stability with increased duration of AF could be observed (150h vs. 193h; 191h vs. 363h; and 200h vs. 479h for (C) and (FC) at short, medium and long AF, respectively) as shown in Table 2.

Table 2. Dry matter loss and aerobic stability (mean $\pm$ std dev) of grass, grass/legume, alfalfa, and corn silages at short (< ~2 weeks), medium (~1 month), or long (2-4 months) fermentation at average 34.7 % DM (20.8-56.3%)

	Short ⁴		% ^b	Medium ⁵		% ^b	Long ⁶		% ^b
	C	FC		C	FC		C	FC	
Duration of fermentation (d)	8.7 (2-16)			31.2 (30-32)			89.8 (60-120)		
Fermentation DM losses (%)	3.0 $\pm$ 1.76	2.9 $\pm$ 1.87	32	5.8 $\pm$ 2.52	4.5 $\pm$ 1.97	81	8.4 $\pm$ 1.88	4.6 $\pm$ 1.67	100
Aerobic stability (hours)	150 $\pm$ 145.5	193 $\pm$ 84.2	92	191 $\pm$ 191.7	363 $\pm$ 171.4	81	200 $\pm$ 142.6	479 $\pm$ 228.1	100

C – non-inoculated; FC – inoculated with *Lactococcus lactis* and *Lentilactobacillus buchneri*. ^b% of trials with significant improvements on traits by applying FC (P < 0.05). ⁴8 contrasts (average 34.6 % DM); ⁵7 contrasts (average 35.1% DM); ⁶15 contrasts (average 34.5% DM).

Conclusions

The combination of *Lactococcus lactis* (DSM11037) and *Lentilactobacillus buchneri* (DSM22501) reduced dry matter loss and improved aerobic stability across all crops, DM concentrations and duration of AF tested, demonstrating the versatility of the silage inoculant.

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Mycotoxdegradome: A Comprehensive Database for Exploring Fungal Mycotoxin-degrading Enzymes and Their Applications

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Keywords: Mycotoxin-degrading enzyme repository, Enzyme annotation, Molecular docking

Introduction

Fungal mycotoxins are critical secondary metabolites produced by certain fungi, posing serious threats to food safety, agricultural sustainability, and human health. Common mycotoxins, including aflatoxins, zearalenone, and fumonisins, have been associated with severe toxicological effects, such as carcinogenicity, immunosuppression, and endocrine disruption. Controlling mycotoxin contamination is a major challenge in global food systems, necessitating innovative and efficient mitigation strategies.

Among the various approaches to address mycotoxin contamination, enzymatic degradation has emerged as a highly promising solution due to its specificity, efficacy, and environmental safety. However, existing research on mycotoxin-degrading enzymes is fragmented, limiting the accessibility of critical information required for advancing scientific discovery and practical applications.

To bridge this gap, we developed MycoToxDegradome, the first comprehensive global database dedicated to fungal mycotoxin-degrading enzymes. This study systematically retrieved and analyzed data from 454 international publications, integrating microbial origins, sequence characteristics, enzymatic properties, and reaction performance into a single platform. By utilizing molecular docking visualization, we also explored enzyme-substrate interaction mechanisms, aiming to provide researchers with actionable insights and analytical tools for advancing food safety and biotechnology.

Materials and methods

1. Data Collection and Annotation

Enzyme data, including ID, name, EC number, sequence, microbial source, properties, and degradation conditions, were extracted from PubMed, Google Scholar, and Web of Science using targeted keywords. Additional sequence and 3D structure data were retrieved from NCBI, UniProt, and PDB (or AlphaFold) with rigorous standardization and quality checks.

2. Molecular Docking and Visualization

Mycotoxin structures were sourced from PubChem or modeled with Chem3D. Enzyme structures from PDB or AlphaFold were preprocessed using PyMOL. AutoDock Vina identified binding modes, key residues, and active sites.

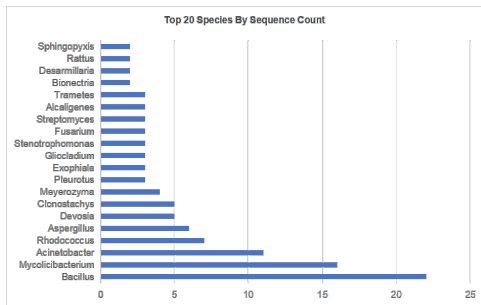
3. Database Construction

Developed with Python (Django), MySQL, and JavaScript, the database integrates enzyme data, supporting efficient queries, filtering, and interactive exploration.

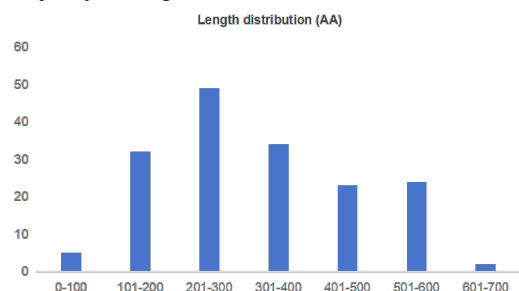
Results

1.Database Overview

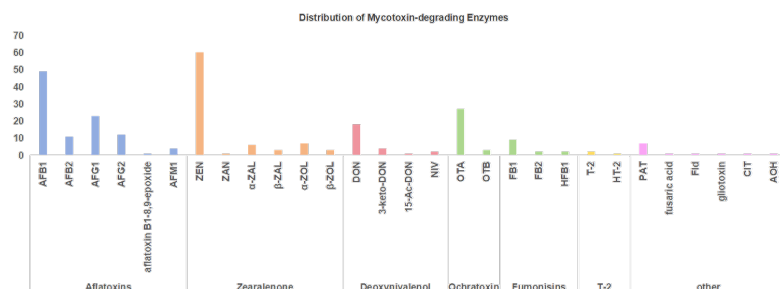
- MycoToxDegradome integrates data from 454 publications, encompassing microbial sources across 7 phylogenetic phyla, 128 genera, and 210 species.
- It annotates 169 enzymes with sequence data, detailing their properties, optimal conditions, and degradation performance. Most enzymes originate from the genera Bacillus, Mycolicibacterium, Acinetobacter, Rhodococcus, and Aspergillus.



- The sequence length of fungal mycotoxin-degrading enzymes ranges from 46 to 695 amino acids, with the majority falling within the 200–300 amino acid range



- Analysis of the distribution of degrading enzymes for various fungal mycotoxins reveals a higher prevalence of enzymes targeting aflatoxins and zearalenone.



2. Enzymatic Characteristics

Most enzymes show optimal activity under mild conditions (pH 6.0–8.0, 25–40°C) with notable substrate specificity and kinetic variations.

3. Insights from Molecular Docking

Docking simulations revealed conserved active site residues involved in substrate binding, providing valuable insights into structure-function relationships.

4. User Accessibility

The platform (<http://82.156.86.35>) offers tools to explore enzyme data, compare performance, and analyze docking results.

Conclusions

MycoToxDegradome advances mycotoxin research by providing a centralized resource on mycotoxin-degrading enzymes. By integrating data on microbial origins, enzyme properties, and interaction mechanisms, it supports innovation in food safety and biotechnology. Molecular docking insights deepen understanding of enzyme-substrate interactions, enabling targeted engineering and industrial applications. The database aims to foster collaboration and drive sustainable solutions for mitigating mycotoxin contamination.

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MycoToxDegradome Is a Curated Database Dedicated to Mycotoxin-degrading Enzymes

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Keywords: Fungal mycotoxins, Mycotoxin-degrading enzymes, Database construction

Introduction

Fungal mycotoxins are critical secondary metabolites produced by certain fungi, posing serious threats to food safety, agricultural sustainability, and human health. Common mycotoxins, including aflatoxins, zearalenone, and fumonisins, have been associated with severe toxicological effects, such as carcinogenicity, immunosuppression, and endocrine disruption. Controlling mycotoxin contamination is a major challenge in global food systems, necessitating innovative and efficient mitigation strategies.

Among the various approaches to address mycotoxin contamination, enzymatic degradation has emerged as a highly promising solution due to its specificity, efficacy, and environmental safety. However, existing research on mycotoxin-degrading enzymes is fragmented, limiting the accessibility of critical information required for advancing scientific discovery and practical applications.

To bridge this gap, we developed MycoToxDegradome, the first comprehensive global database dedicated to fungal mycotoxin-degrading enzymes. This study systematically retrieved and analyzed data from 454 international publications, integrating microbial origins, sequence characteristics, enzymatic properties, and reaction performance into a single platform. By utilizing molecular docking visualization, we also explored enzyme-substrate interaction mechanisms, aiming to provide researchers with actionable insights and analytical tools for advancing food safety and biotechnology.

Materials and methods

Data Collection and Annotation

A systematic literature retrieval was conducted using major scientific databases (e.g., PubMed, Web of Science, Scopus) with keywords such as "mycotoxin degradation," "fungal mycotoxins," and "enzymatic detoxification."

Publications were screened to identify studies reporting mycotoxin-degrading enzymes. Key information, including microbial sources, enzyme sequences, enzymatic properties, thermal stability, pH tolerance, and degradation conditions, was extracted and standardized.

Database Construction

The collected data were curated into a relational database structure, ensuring comprehensive annotation and interlinking of enzyme attributes.

Data processing pipelines were developed for sequence validation, taxonomic classification, and physicochemical property prediction using bioinformatics tools such as BLAST, ClustalW, and ProtParam.

Molecular Docking and Visualization

Molecular docking analyses were performed using AutoDock Vina to investigate the interaction mechanisms between enzymes and mycotoxin substrates.

Structural visualizations were created using PyMOL and Chimera to highlight key binding residues and active site features.

Database Interface Development

The database interface was developed using modern web technologies (e.g., Django, React) to enable interactive data browsing and visualization.

Analytical tools, including sequence search and degradation performance comparison, were implemented to enhance user accessibility and utility.

Results

Database Overview

MycoToxDegradome integrates data from 454 publications, covering microbial sources across 7 phylogenetic phyla, 128 genera, and 210 species.

A total of 169 enzymes with sequence data were annotated, with detailed information on their enzymatic properties, optimal reaction conditions, and degradation performance.

Enzymatic Characteristics

The majority of enzymes exhibited optimal activity under mild conditions (pH 6.0–8.0, 25–40°C), with significant variability in substrate specificity and reaction kinetics.

Thermal stability and pH tolerance profiles highlighted the potential for industrial applications in diverse environmental settings.

Molecular Docking Insights

Docking simulations revealed conserved active site residues involved in substrate binding, providing insights into structure-function relationships.

Enzymes from bacterial and fungal sources demonstrated distinct interaction patterns, suggesting phylogenetic influences on catalytic mechanisms.

User Accessibility

The online platform (<http://82.156.86.35>) features interactive visualization tools, enabling researchers to explore enzyme data, compare degradation performance, and analyze molecular docking results.

Conclusions

MycoToxDegradome represents a significant advancement in the field of mycotoxin research, offering a centralized resource for exploring and utilizing mycotoxin-degrading enzymes. By systematically integrating data on microbial origins, enzymatic properties, and interaction mechanisms, the database provides a robust platform for driving scientific innovation in food safety and biotechnology. The molecular docking insights further enhance our understanding of enzyme-substrate interactions, paving the way for targeted enzyme engineering and industrial applications. We envision MycoToxDegradome catalyzing collaborative research efforts and contributing to the development of sustainable solutions for mitigating mycotoxin contamination globally.

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Oxytetracycline's Impact on Corn Silage Microbes and ARGs: Metagenomic and Metatranscriptomic Study

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Keywords: Oxytetracycline, ARGs, microbial communities, metagenomic, metatranscriptomic

Introduction

Residual antibiotics excreted in animal feces are introduced into the soil, from which they are transferred to plants through various mechanisms (Huang et al., 2021). These crops, subsequently used for silage production, often contain numerous antibiotic resistance genes (ARGs). Despite the significance of this process, there is a lack of comprehensive studies on the microbial communities and ARGs present in silage as a result of this transfer. Addressing this cycle is crucial for mitigating the spread of antibiotic resistance.

Materials and methods

Freshly harvested and chopped whole crop corn (*Zea mays* L.) from Wuwei City, Gansu Province, China was divided into eighteen subsamples. Six of the subsamples were randomly assigned to one of the oxytetracycline (OTC) concentration treatments (0, 40 and 80 µg/kg fresh weight) and ensiled for 3 and 90 days in a vacuum-sealed polyethylene bags (30 × 23 cm). At each ensiling period, three replicates for each treatment were opened and samples were taken for OTC residual, metagenomic and metatranscriptomic analyses. Subsequently, microbial community analyses as well as ARGs annotation were conducted to track microbial succession, viable microbiota, ARGs and their expression.

Results

The OTC has been degraded during the fermentation process, with a degradation rate of 55% in the control group and 85% in the OTC treatment group after 90 days of fermentation, while it had little effect on microbial diversity. *Weissella*, *Leuconostoc*, *Lactococcus*, and *Pediococcus* were shown to be high-abundance bacteria in pre-fermentation detected by metagenomic (41.5%) and metatranscriptomic (86.2%). Metagenomic also discovered 17.7% unidentified and 39.5% undesirable bacteria. Both approaches identified *Lactiplantibacillus*, *Weissella*, *Levilactobacillus*, *Companilactobacillus*, *Pediococcus*, *Leuconostoc*, and *Loigolactobacillus* after three days of ensiling, which accounted for 96.3% of the metatranscriptomic and 78% of the metagenomic. The largest difference in composition after 90 days was found that there were majority of bacteria identified by metagenomic (52%–85%) belonged to the seven aforementioned genera, while *Lentilactobacillus* dominated the relative abundance of metatranscriptomic (51%–88%).

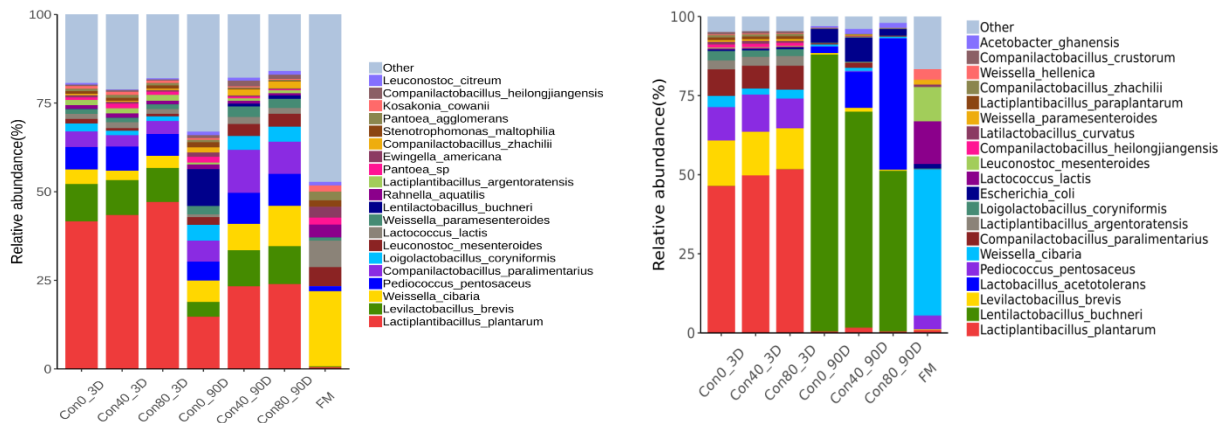


Fig 1 Bacterial community dissimilarities of corn silage.(Left, metagenomic detected; right, metatranscriptomic detected)

The most abundant ARGs in corn silage were *bacA*, *tetM*, and *InuA* from metagenomics while there were much more *tetC*, *APH(3')-Ia* and *bacA* were expressed during ensiling. And most expressed ARGs could resistant tetracyclines, novobiocin, bacteriocin, macrolide lincosamide streptomycin (MLS), multidrug resistance, and aminoglycosides. Relative expression, which indicates how much a gene was verexpressed or underexpressed in the metatranscriptomic relative to the metagenomic, is calculated by dividing its relative activity by its relative abundance. After fermentation, most ARGs' relative expression decreased except *ermB*. After 90 days, other major facilitator superfamily transporters' relative expression increased in control and 80 ug/kg groups. High-risk ARGs like *tetM*, *ermB*, *APH(3')-IIIa* had high relative expression before fermentation, indicating potential increase after fermentation. After fermentation, most high-risk resistance genes' relative expression significantly decreased or were undetectable. After 3 days, only *ermB*, *APH(3')-IIIa*, etc. showed high expression. 80 ug/kg group promoted *APH(6')-Ib*, 40 ug/kg group promoted *InuB*. After 90 days, only

APH(3')-Ia, catB11 showed high expression. 80 ug/kg group reduced APH(3')-Ia but increased mphB, 40 ug/kg group promoted catB11 and ANT(2'')-Ia.

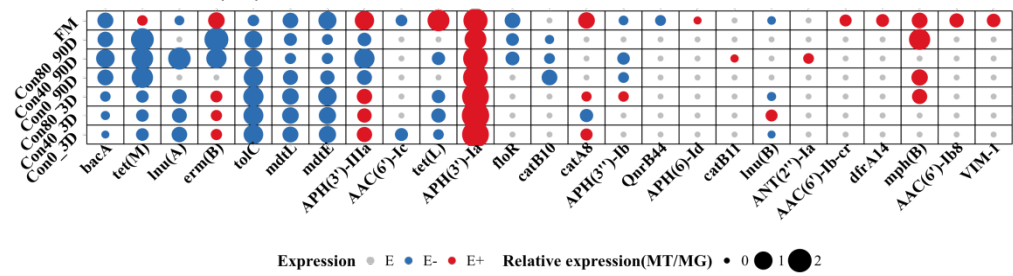


Fig 2 Relative expression of high-risk ARGs

Tetracycline-related ARGs in DNA and RNA were significantly impacted by OTC degradation, fermentation duration, and severe OTC addition. Pathogenic bacteria such as *Klebsiella*, *Enterobacter*, *Kosakonia*, *Pantoea*, *Serratia*, and others were the primary inhabitants of ARG hosts. ARGs were also hosted by *Lactococcus lactis* and *Pediococcus oentoseus*, however their availability were greater prior to and following three days of fermentation and falls as fermentation progresses.

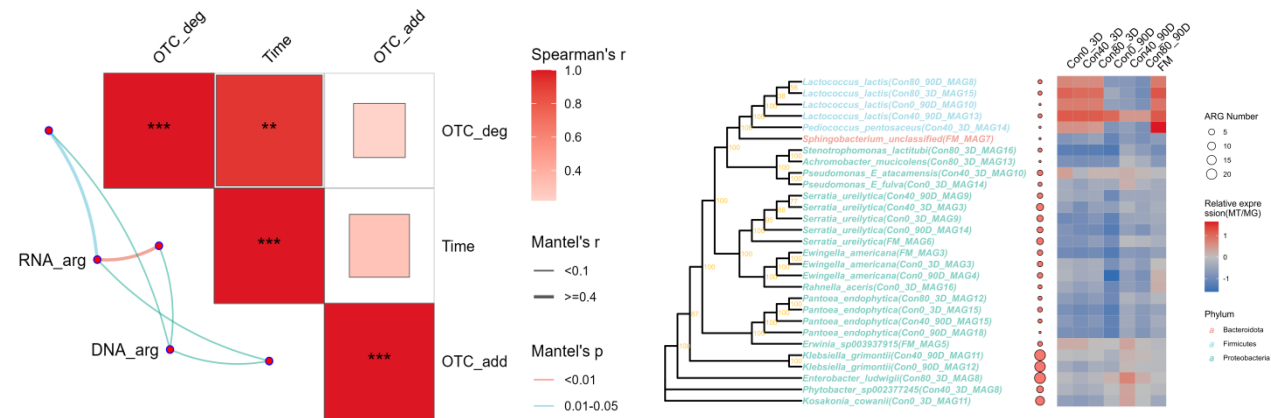


Fig 3 Effects of OTC, it's degradation and fermentation time on tetracycline related ARG composition (left) and ARG hosts (right)

Conclusions

The OTC could be degraded during the fermentation process, while it had little effect on microbial diversity. This study firstly revealed the functional viable bacteria during ensiling by metatranscriptomic technology and the largest difference in composition after 90 days was found. Fermentation reduced the antibiotic resistance by lowered the relative expression levels of most ARGs, particularly high-risk ARGs. In addition, fermentation significantly affected tetracycline related ARGs, reduced potential resistance gene hosts.

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Effects of Antifungal and Detoxifying Lactic Acid Bacteria on the Fermentation Quality, Mycotoxin Content, Microbial Community and Functional Changes of Silage Corn

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Keywords: Whole plant corn silage; Mycotoxins; Fermentation quality; Metagenomics

Introduction

As global warming progresses, extreme weather events have been occurring with greater frequency. Extreme climates such as persistent drought, high temperatures, and floods have substantially heightened the susceptibility of crops to mold infections. Consequently, a growing number of agricultural regions are witnessing their crops being tainted by molds such as *Aspergillus flavus* and *Fusarium* (Ogunade et al, 2018). The invasion of molds during the field growth not only affects crop yields but also results in the accumulation of mycotoxins, seriously threatening food and feed security (Gallo et al, 2021). The growth period of corn coincides with the season of high temperature and heavy rainfall, making it vulnerable to mold invasion. Correspondingly, the mycotoxin contamination of whole-plant corn silage will affect livestock production and the subsequent safety of livestock products. Therefore, there is an urgent need to develop targeted key technologies for the prevention and control of mycotoxin contamination in silage. This study aimed to evaluate the effects of 5 LAB strains with both antibacterial and detoxification functions on the fermentation quality, mycotoxin content, microbial community, and functional changes of whole plant corn silages.

Materials and methods

The fungal (FI) infected or non-fungal infected (NFI) corn ensiled with deionized water (uninoculated) or *A. tropicalis* AT7, *Lp. plantarum* LP15, *Lp. plantarum* LP20, *E. faecium* Y83, *E. faecalis* F85. The inoculants were applied each at about 1.0×10^6 cfu g⁻¹. Then samples were collected for analysis after 5, 14, and 90 days of ensiling. The concentrations of mycotoxins in silages were quantified using an Ultra-performance Liquid Chromatography-tandem Mass Spectrometry system, and bacterial and fungal community were analysed using the Illumina MiSeq PE300 platform according to Bao et al. (2024). The data were analysed by two-way ANOVA with the fixed effects of inoculants, fungal infestation and their interaction using the GLM procedure of the Statistical Analysis System (SAS 9.3). Tukey's HSD test was used to compare the differences between the treatment means and the significance was declared at $P < 0.05$.

Results

The results showed that inoculated *Lp. plantarum* LP15, *Lp. plantarum* LP20, and *E. Faecium* Y83 increased LA content in NFI silages. Regardless of fungal infection, inoculating *E. faecalis* JF85 reduced ethanol content. Fungal infection increased the contents of AFB1 and DON, while inoculating *Lp. Plantarum* LP15 reduced ZEN content. Fungal infection increased the abundance of *Lp. plantarum*, inoculating with *A. tropicalis* AT7 increased the abundance of *Pediococcus pentosaceus*, inoculating *Lp. Plantarum* LP15 increased the abundance of *Lp. plantarum*, and inoculating *E. Faecium* Y83 increased the abundance of *Pediococcus acidilactic* and *Lactocaseibacillus rhamnosus*. In NFI group, *Candida tropicalis*, *Candida glabrata*, and *Pichia kudriavzevizi* were the dominated fungi.

Conclusions

Fungal infection reduced the fermentation quality and increased mycotoxins content of whole-plant corn silage, and promoted the metabolism of carbohydrates and amino acids. Inoculating with *Lp. plantarum* LP15 reduced ZEN content. Inoculating *A. tropicalis* AT7, *Lp. plantarum* LP15 and *E. faecium* Y83 promoted the proliferation of homo-fermentative LAB and reduced the abundance of genes related to acetic acid synthesis, and effectively alleviated the adverse effects of fungal infection on the whole-plant corn silage.

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Small Scale Corn Silage for Quality Fodder Conservation in the Integrated Crop-livestock Systems of the Sahel

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Key words: corn silage, djalonke sheep, crop-livestock system, forage and Sahel.

Introduction

Adoption of appropriate forage conservation technics in the crop-livestock systems of Sahel has a potential of increasing availability of quality fodder for ruminant during the long dry season during. For this purpose, a small-scale corn silage making technic was developed and tested for local Djalonke sheep fattening in Burkina Faso.

Materials and methods

The work was carried out on farms in the southern Sudanian zone of Burkina Faso, with local farmers who mainly practiced rainfed corn and sorghum production as stable food and the straw being used as fodder for their animals. The forage used for making the silage in this study was fresh corn fodder harvested at grain maturity. Other materials used included a shopper, triple-bottomed silage bags, pestles and plastic films. The fresh forage obtained from field after harvesting the corn cobs, was chopped and pre-wilted, then progressively filled and compacted in the silage bags, and finally covered with plastic film and hermetically sealed. The sealed silage bags are then stored in appropriate place and the silage will be ready for use from the 2nd month, and can be conserved up to six months. The silage produced nutritional quality, effect on sheep weight gain in fattening and it's preference by local farmers were compared in a trial to the traditional farmers practice of corn straw conservation and utilization the study area.

Results

The results showed that the small-scale corn silage developed, has higher crude protein concentration (6.41% vs 4.14%), and improve the *in vitro* digestibility (46.11% vs 41.90%) of the corn fodder as compared to the traditional straw conservation technic. In addition, djalonke sheep fed with the corn silage had the best daily weight gain (133.33 g.day⁻¹) and allows better gross margin (18 100 FCFA) than the traditional technic (12 100 FCFA) after two months fattening. We conclude that this small-scale silage making technic is can be scaled within the small holder livestock keeper's community in Burkina Faso to increase availability and utilization of quality fodder for their animals.

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Effects of Enzymes, Microbiota and Organic Acids on Structural Carbohydrate During Ensiling of Sudan Grass

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Keywords: enzymatic degradation, microbial degradation, acid degradation, structural carbohydrate, silage.

Introduction

Ensiling is a crucial technique for preserving the nutritional value of green forage. However, for tropical forages, the high fiber concentration and low water-soluble carbohydrate concentration pose significant challenges in making high-quality silage. To address these challenges, an effective approach is to increase WSC concentration by degrading structural carbohydrates. Meanwhile, the degradation of structural carbohydrate can reduce the crude fiber concentration of silage, thereby improving its palatability and digestibility. Typically, the degradation of structural carbohydrates during ensiling involves three primary mechanisms: enzymatic degradation, microbial degradation, organic acid degradation. This study investigates the effects of these three degradation pathways on the structural carbohydrates of Sudan grass silage, which can guide new additive development and silage modulation strategies.

Materials and methods

Sudan grass was harvested in the filling stage and the dry matter concentration was 222 g/kg DM. Three silage bags (20 × 25 cm) for each treatment were filled with 100g of grass. The silage bags were stored at room temperature (24-27°C) and opened for subsequent analyses after 60 days of ensiling. In this study, γ-ray irradiation and short-term high temperature enzyme deactivation technology were used to eliminate the influence of microorganisms and plant endogenous enzymes, respectively. Using microbiota transplantation technology to transfer the surface microbiota. Control group (CON): γ-ray pre-sterilized + enzyme deactivation, ED group: γ-ray pre-sterilized, MBD group: γ-ray pre-sterilized + epiphytic microbiota, OAD group: γ-ray pre-sterilized + enzyme deactivation + organic acid mixture. The organic acid mixture was mixed of lactic acid, acetic acid, propionic acid in a ratio of 8.5:1.3:0.2 (v/v). The data were analyzed using one-way analysis of variance (ANOVA) with GLM in SPSS.

Results

Compared to the CON group, the MBD group exhibited the lowest pH (3.61) and the highest lactic acid concentration (110 g/kg DM). The water-soluble carbohydrate concentrations in ED group (128.67 g/kg DM) and OAD group (165 g/kg DM) was higher than that in CON group. The concentrations of neutral detergent fiber, acid detergent fiber and acid detergent lignin in the ED, MBD and OAD group were lower than those in the CON. Compared to the CON group, cellulose and hemicellulose concentrations decreased to varying degrees in all groups, the MBD group showing the lowest concentrations (247.53 and 219.67 g/kg DM, respectively), followed by ED group (253.33 and 223 g/kg DM). The lignocellulose degradation rate in the MBD was the highest among all groups. The OAD group had the highest fructose and xylose concentrations (55.86 and 42.25 g/kg DM, respectively), while the MBD group exhibited the highest glucose concentration (7.02 g/kg DM).

Conclusions

The above results indicate that epiphytic microbiota made the most significant contribution to the degradation of structural carbohydrates, followed by enzymes, with organic acids having the least impact. In the future, the development of silage additives for high-fiber and low-sugar material should focus more on microbial preparations.

Table 1. Effects of degradation mode on fermentation characteristics of Sudangrass silages after 60 days of ensiling.

Item	CON	ED	MBD	OAD	SEM	P-value
Dry matter, g/kg FM	232.88	237.01	227.06	247.84	6.930	0.184
Water-soluble carbohydrate, g/kg DM	124.00b	128.67b	35.37c	165.00a	15.840	<0.001
pH	5.28b	5.36a	3.61d	4.41c	0.250	<0.001
Lactic acid, g/kg DM	23.60b	5.10b	110.00a	9.94b	12.690	<0.001
Fructose, g/kg DM	51.90a	39.82a	5.89b	55.86a	6.270	<0.001
Xylose, g/kg DM	34.75	7.33	7.63	42.25	6.450	0.087
Glucose, g/kg DM	6.66	6.02	7.02	5.61	0.280	0.327
Sucrose, g/kg DM	8.70a	5.05ab	3.71ab	2.27b	0.890	0.032

Means with different letters indicate significant differences among treatments over the same time period. SEM, standard error of means. DM, dry matter; FM, fresh matter.

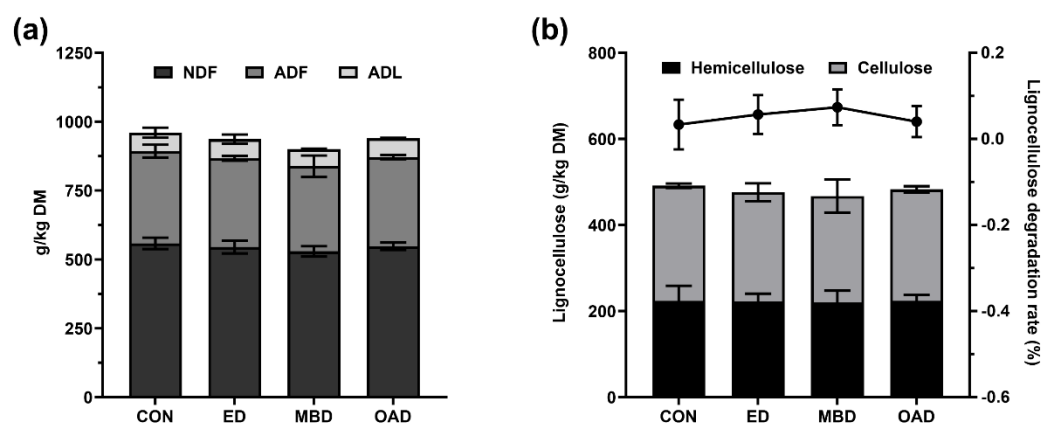


Figure 1. The concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (a). The concentrations of cellulose and hemicellulose and the degradation rate of lignocellulose (b).

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Reduction of Pathogenic Bacteria from Source Through Stubble Height and Silage Management of *Pennisetum giganteum*

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Keywords: *Pennisetum giganteum*, stubble height, silage, microbial community, pathogenic risk

Introduction

The demand for animal products (meat and milk) worldwide has been on the rise as consumption levels improve, leading to new requirements in terms of economic and safety standards. Ensuring the safety of herbage or silage is crucial for producing healthy ruminant foods. Pre-harvest management practices, such as stubble height, can influence the chemical and nutritive composition of herbage. In practical production, it is common to select stubble height randomly for silage making, and this lack of emphasis on stubble height selection is also reflected in silage studies. It is important to recognize the significance of stubble height, as low stubble height may elevate the risk of microbial contamination and pathogen invasion (Ogunade et al., 2018). However, there is limited literature exploring the effect of stubble height and fermentation period on the silage safety. Previous studies emphasized the need to consider phenotypes, particularly pathogenic potential, in the analysis of microbial communities (Ward et al., 2017). By integrating phenotype prediction with sequencing data by BugBase tool, researchers can unlock valuable information that contributes to the field of silage research and aids in addressing important questions related to microbial ecology and pathogenicity. Therefore, this study aimed to evaluate the effects of stubble height and fermentation time on the fermentation products, microbial community, and pathogenic risk of *P. giganteum* silage.

Materials and methods

The *P. giganteum* is managed as recommended with irrigation and fertilizer (organic fertilizer, 15 t/ha at tillering stage) during growth and no fertilization 30 days before cutting. Under the recommended growth stage (late vegetative stage), *P. giganteum* was harvested at stubble heights of 15 cm (R₁₅) or 45 cm (R₄₅). The moist and rainy climate of the harvest area makes withering difficult to proceed. Approximately 0.45 kg of R₁₅ or R₄₅ was placed into a laboratory silo (280 × 350 mm polythene bag), vacuum-sealed, and stored at environment temperature (22.0±4°C). After ensiling for 2, 4, 8, 16, 32 and 64 days, six silos per treatment at each sampling day were opened for fermentation quality, bacterial community, and pathogenic risk analyses. Total genomic DNA was extracted using the Kit and the near full length of the bacterial 16S rRNA gene was amplified with primers 27F/1492R and sequenced on Pacbio Sequel II System. The BugBase tool were employed for predicting pathogenic risk, respectively. Differences between stubble heights were processed by t-test, and data on fermentation parameters were processed by two-way ANOVA with stubble height and fermentation duration as fixed effects (SAS 9.2).

Results

The increase of stubble height from 15 cm to 45 cm significantly ($P < 0.05$) increase the crude protein content, but significantly ($P < 0.05$) decreased the dry matter (DM) and water soluble carbohydrate (WSC) content and aerobic bacteria number of fresh *P. giganteum* (data not shown due to space constraints). After 64 days of ensiling, both stubble heights presented desirable fermentation, as characterized by high lactic acid concentration and lactic/acetic acid (> 3.0), low pH value (< 4.2) and ammonia nitrogen concentration (< 100 g/kg TN) as well as undetected butyric acid (Table 1). As presented in Figure 1a, the pathogenic contribution analysis highlighted *Acinetobacter* and *Serratia* as major contributors to the pathogenic risk of R₁₅. *P. giganteum* harvested at low stubble height had significantly ($P < 0.05$) greater proportions of bacterial 'Potentially Pathogenic' than *P. giganteum* harvested at high stubble height, while these undesirable phenotypes declined to negligible levels after ensilage and no differences ($P > 0.05$) between stubble heights were observed (Figure 1b).

Table 1. Effects of stubble height and fermentation duration on fermentation parameters (g/kg DM) of *P. giganteum* silage.

Items	Stubble height	Fermentation duration (day)						SEM	P value		
		2	4	8	16	32	64		SH	FD	SH×FD
pH	R ₁₅	5.21 ^A	4.61	3.87	3.45	3.44	3.53	0.102	0.001	<0.001	0.009
	R ₄₅	4.86 ^B	4.31	3.71	3.50	3.40	3.54				
DM (g/kg FW)	R ₁₅	190 ^A	187	187	188 ^A	182	178 ^A	1.854	<0.001	0.289	0.989
	R ₄₅	171 ^B	172	172	169 ^B	168	163 ^B				
WSC	R ₁₅	141 ^A	130	123	106	69.2	78.3	4.890	0.089	<0.001	0.799
	R ₄₅	125 ^B	112	103	82.2	69.8	82.0				
Lactic acid	R ₁₅	0.05	6.96	21.8 ^B	48.4	59.3 ^B	54.6	4.458	<0.001	<0.001	0.093
	R ₄₅	3.33	8.35	30.8 ^A	57.9	77.5 ^A	66.6				
Acetic acid	R ₁₅	0.06 ^B	6.74	11.3	9.85	18.1	17.0	1.135	0.006	<0.001	0.511
	R ₄₅	2.58 ^A	5.79	13.9	13.8	21.7	19.1				
Lactic/acetic acid	R ₁₅	0.35 ^B	1.11	1.96	5.20	3.28	3.32	0.241	0.331	<0.001	0.102
	R ₄₅	1.34 ^A	1.46	2.25	4.19	3.57	3.51				
Butyric acid	R ₁₅	ND	ND	ND	ND	ND	ND	--	--	--	--
	R ₄₅	ND	ND	ND	ND	ND	ND				
Ethanol	R ₁₅	ND	0.02	0.20	ND	4.39	0.63	--	--	--	--
	R ₄₅	ND	ND	0.04	ND	3.08	0.67				
NH ₃ -N (g/kg TN)	R ₁₅	39.9	61.3 ^A	67.1 ^A	71.6	89.4 ^A	98.0 ^A	3.083	<0.001	<0.001	0.037
	R ₄₅	41.2	43.7 ^B	51.1 ^B	62.9	63.4 ^B	75.6 ^B				

^{A-B} Distinct letters within the same row indicate statistically significant differences at a significance level of $P < 0.05$.

FW, fresh weight; TN, total nitrogen; ND, non-detectable; SEM, standard error of means.

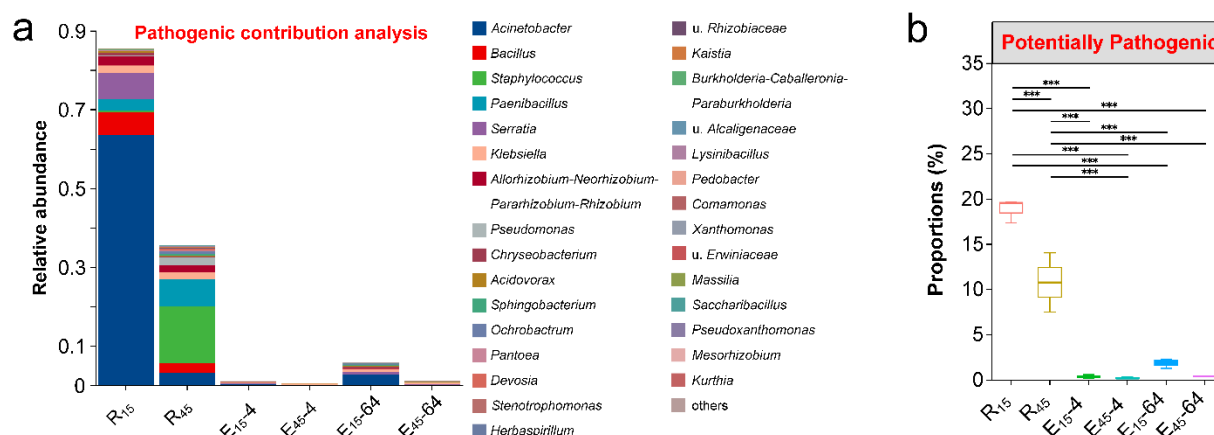


Figure 1. Pathogenic risk of raw *P. giganteum* and *P. giganteum* silage produced at various stubble heights and fermentation durations. (a) Bar plot of pathogenic contribution analysis. (b) Box plots of Potentially Pathogenic. Significant results of $P < 0.001$ are denoted by "****".

Conclusions

Stubble height, and to a greater degree, fermentation time had significant influences on the fermentation products, bacterial community diversities and pathogenic risk of *P. giganteum* silages. Concerns about the risk of microbial contamination and pathogens from low stubble height (e.g. 15 cm) could be alleviated by ensiling.

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Comparison of M5 Agar and MRS Agar for Differentiation of *L. plantarum* and *L. buchneri* in Silage Additives

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Keywords: Homofermentative bacteria, heterofermentative bacteria, *L. buchneri*, *L. plantarum*, silage additive, determination method.

Introduction

Lentilactobacillus buchneri (*L. buchneri*) is commonly used in silage additives to improve aerobic stability of ensiled material (Kung and Ranjit, 2001). In silage additives containing *L. buchneri* and *Lactiplantibacillus plantarum* (*L. plantarum*), a differential determination of cell counts in the product is desirable. According to ISO 15214:1998 standard (Technical Committee ISO/TC 34, 1998), MRS agar should be used for determination of cell counts of lactic acid bacteria. To differentiate between homo- and heterofermentative lactic acid bacteria, the M5 agar has been developed (Zúñiga et al., 1993). The aim of this study was to compare differential determination of cell count in a silage additive containing *L. buchneri* and *L. plantarum* using M5 or MRS agar.

Materials and methods

M5 agar was prepared according to Zúñiga et al. (1993). MRS agar plates were ready-to-use plates (BIOSOLUTE®, Th. Geyer GmbH & So KG, Renningen, Germany). Three different batches of a mixture of *L. buchneri* (DSM 34837, 85%) and *L. plantarum* (DSM 8866, 7.5% and DSM 8862, 7.5%) and three different bags (20g) each of those batches were used.

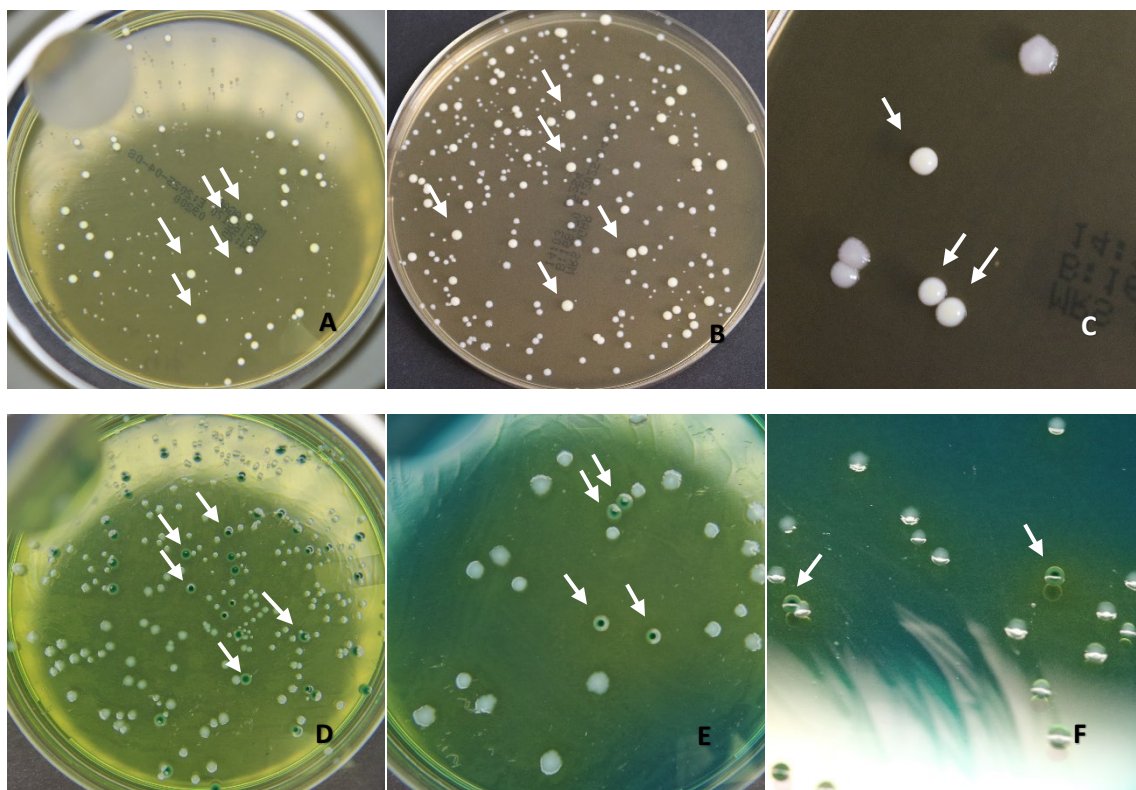


Figure 14 Pictures of *L. plantarum* and *L. buchneri*. Upper row: MRS agar incubated at 30°C for 48h (A) and 37°C for 48h (B) and at 37°C for 72h (C). A: large white colonies are *L. plantarum*. *L. buchneri* colonies grow slowly and are barely visible; B: at 37°C, *L. buchneri* colonies grow faster and the difference in size is smaller. C: after 72h at 37°C, *L. buchneri* colonies have an irregular margin, greyish colour and are slightly larger than *L. plantarum*. Bottom row: M5 agar incubated at 37°C for 48h (D) and at 37°C for 72h (E and F). D: *L. plantarum* can be differentiated by dark blue-green colour of colonies; E: after 72h at 37°C, *L. plantarum* colonies are translucent and blue-green, flat and round, *L. buchneri* colonies are translucent, greyish, flat, with an irregular margin. F: on M5 agar, *L. buchneri* and *L. plantarum* colonies appear translucent if plate is held at an angle. *L. plantarum* are indicated by white arrows.

Samples were prepared and diluted according to ISO 15214:1998 and plated in duplicate by spread plate technique. Dilutions 10^{-9} and 10^{-10} were plated. M5 agar plates (dilutions 10^{-9} and 10^{-10}) and MRS agar plates (dilution 10^{-10}) were incubated at 37°C for 72h. The remaining MRS agar plates (dilution 10^{-9}) were incubated at 30°C for 72h. Plates were read at 48h and 72h. On M5 agar plates, colonies of homofermentative *L. plantarum* appear dark blue-green, flat, and

smooth, whereas heterofermentative *L. buchneri* colonies appear greyish, translucent, flat and irregular (Figure 1). On MRS agar plates, *L. buchneri* colonies appear greyish-beige with a rough margin and slow growth at 30°C and fast growth at 37°C. *L. plantarum* colonies grow fast and are circular, convex, beige, and opaque with a smooth margin.

Results

On MRS agar incubated at 30°C and read after 48h with dilution 10^{-9} , *L. plantarum* colonies have a diameter of approximately 1-1.5 mm with round, convex, opaque beige colony morphology, whereas *L. buchneri* colonies have a diameter of about 0.2 mm and are transparent. At 37°C *L. plantarum* colonies look similar to 30°C, however, *L. buchneri* colonies are larger at about 1 mm diameter with a rough margin and translucent, greyish colony morphology. At 37°C after 72h using MRS agar, *L. buchneri* and *L. plantarum* colonies are difficult to differentiate by size but only by colony morphology. Total colony count and count of *L. buchneri* and *L. plantarum* colonies did not differ between methods (Table 1).

Table 1. Cell count of *L. buchneri* and *L. plantarum* on MRS and M5 agar at 30°C or 37°C incubation temperature.

Colony forming units (CFU)	MRS		M5		Average difference		P-Value
	mean	SD	mean	SD	Cell count	%	
48h							
Total CFU ¹	3.1*10 ¹¹ (37°C)	4.2*10 ¹⁰	3.1*10 ¹¹ (37°C)	4.6*10 ¹⁰	-1.3*10 ⁹	-0.41	0.95
<i>L. plantarum</i> ²	4.4*10 ¹⁰ (30°C)	5.0*10 ⁹	4.4*10 ¹⁰ (37°C)	3.6*10 ⁹	-3.0*10 ⁸	-0.69	0.90
<i>L. buchneri</i> ¹	2.7*10 ¹¹ (37°C)	4.3*10 ¹⁰	2.7*10 ¹¹ (37°C)	4.5*10 ¹⁰	-9.7*10 ⁸	-0.36	0.96
72h							
Total CFU ¹	3.2*10 ¹¹ (37°C)	4.1*10 ¹⁰	3.2*10 ¹¹ (37°C)	4.2*10 ¹⁰	7.0*10 ⁹	2.17	0.69
<i>L. plantarum</i> ²	4.3*10 ¹⁰ (30°C)	4.8*10 ⁹	4.4*10 ¹⁰ (37°C)	3.3*10 ⁹	-9.7*10 ⁸	-2.24	0.67
<i>L. buchneri</i> ¹	2.8*10 ¹¹ (37°C)	4.2*10 ¹⁰	2.7*10 ¹¹ (37°C)	4.2*10 ¹⁰	8.0*10 ⁹	2.85	0.67

¹ determined at dilution 10^{-10} , ² determined at dilution 10^{-9}

Conclusions

Differentiation of *L. buchneri* and *L. plantarum* colonies on MRS agar incubated at 30°C for 48h is easily possible due to different growth characteristics and colony morphology. At 37°C for 48h, colonies resemble each other in size but differ in colony morphology and still allow for differential determination of cell count. Therefore, the use of MRS agar (counted at 48h and 72h and incubated at 30°C and 37°C) is suitable for differential cell count of *L. buchneri* and *L. plantarum* by differentiation of colony morphology and growth characteristics.

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Effect of Varying Proportions of *L. buchneri* and *L. plantarum* in Silage Additives

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Keywords: Homofermentative bacteria, heterofermentative bacteria, *L. buchneri*, *L. plantarum*, silage additive.

Introduction

Heterofermentative bacteria (e.g. *Lentilactobacillus buchneri* (*L. buchneri*)) produce lactic acid and acetic acid, where the latter is known to improve aerobic stability of silage (Danner et al., 2003). Many companies have developed silage additives using heterofermentative bacteria to increase acetic acid concentration and aerobic stability, however, purely heterolactic fermentation can have disadvantages in terms of decreased silage quality characteristics. To date, it is unclear how well different traits of homo- and heterofermentative bacteria develop when varying proportions of *L. buchneri* and very competitive *Lactiplantibacillus plantarum* (*L. plantarum*). The aim of this study was to determine an ideal combination of *L. buchneri* and *L. plantarum* to improve silage quality and aerobic stability.

Materials and methods

A silage trial in glass jars was conducted over 90 days including 6 groups with different silage additives: 1 = control without additives; 2 = 94% *L. buchneri*, 6% *L. plantarum*; 3 = 85% *L. buchneri*, 15% *L. plantarum*; 4 = 70% *L. buchneri*, 30% *L. plantarum*; 5 = 50% *L. buchneri*, 50% *L. plantarum*; 6 = 100% *L. plantarum*. Perennial ryegrass (*Lolium perenne*) and oat-grass (*Arrhenatherum elatius*) was ensiled with a sugar concentration of 5.5% in FM and a dry matter (DM) content of 39%. All silage additives (groups 2-6) were applied at a concentration of 300.000 CFU / g fresh matter (FM). On days 4 (0.5 L jars and 3 repetitions per group), 11 (0.5 L jars and 3 repetitions per group), 49 (1.5 L jars, 750g FM and 3 repetitions per group), and 90 (1.5 L jars, 750g FM and 5 repetitions per group in groups 1-5 and 3 repetitions in group 6), pH-value of silage was measured. On day 90 after ensiling, pH-value, lactic acid, acetic acid, 1, 2-propanediol, ethanol, butyric acid, propionic acid, weight loss, metabolizable energy (ME), NH₃-N of total N (%) and aerobic stability were assessed. Differences among groups were assessed using Kruskal-Wallis Test and differences between treated groups and the control group were established using Mann-Whitney-U Test.

Results

On days 4 and 11, highest pH-values were observed in groups 1 to 3 and lowest values in group 6 (Figure 1). On day 90, silage additive treatments significantly affected pH-value ($P = 0.002$), concentrations of acetic acid ($P < 0.001$), 1,2-propanediol ($P < 0.001$), and ethanol ($P = 0.001$) as well as weight loss ($P < 0.001$), NH₃-N of total N (%) ($P = 0.02$), and aerobic stability ($P = 0.002$, Table 1). No effect was observed on lactic acid concentration ($P = 0.8$) and ME ($P = 0.5$). Propionic acid and butyric acid were below detection limits. The pH-value was lowest in group 5 and 6 and highest in group 1 (Table 1). Acetic acid and ethanol concentrations were lowest in group 6 and highest in group 1 (ethanol) or 2 (acetic acid). Aerobic stability was highest in group 2 and lowest in group 5. Generally, pH-value, weight loss, concentrations of ethanol, acetic acid, and 1, 2-propanediol, and aerobic stability decreased with increasing proportion of *L. plantarum* in the additive. Aerobic stability in silages with treatments 2 and 3 was significantly higher than the control group. Aerobic stability with treatments 4 ($P = 0.310$) and 6 ($P = 0.146$) was not significantly different from the control group and aerobic stability with treatment 5 was significantly lower than in the control group ($P = 0.016$).

Conclusions

Fermentation characteristics in silages from a mixture of homo- and heterofermentative bacteria can be directly influenced by proportions of bacterial species. A purely homofermentative silage additive has positive effects on silage quality in terms of pH-value, weight loss and low acetic acid and ethanol concentrations, but no advantage in terms of aerobic stability. If an increased aerobic stability is desired, the effect of heterofermentative bacteria is determined by the proportions of bacterial species in the silage additive and the competitiveness of each species.

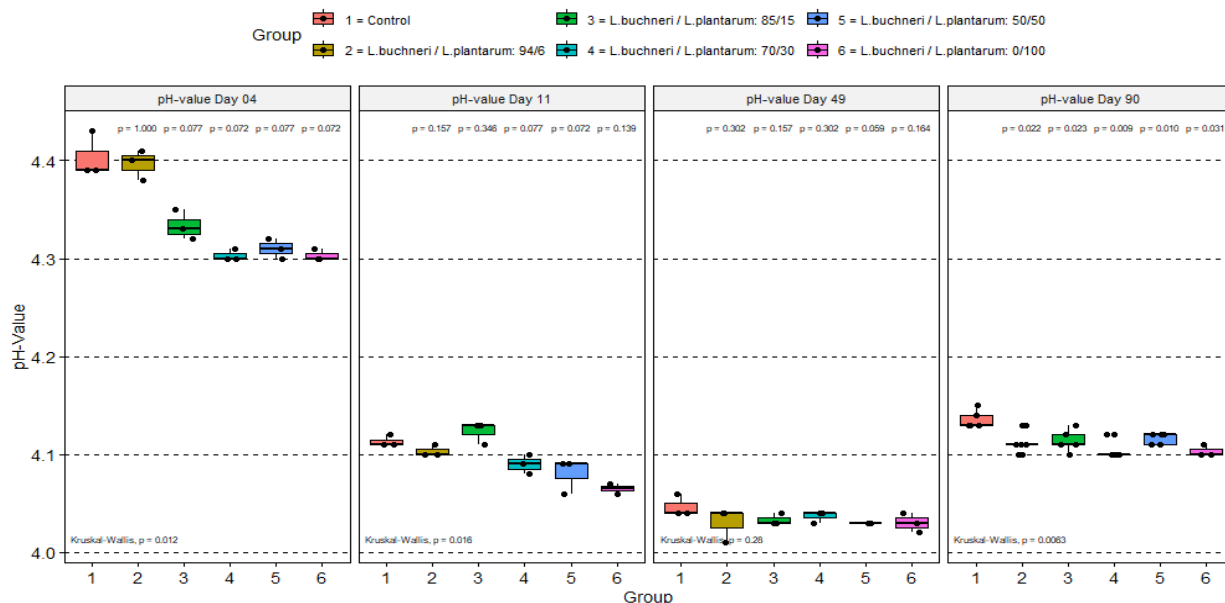


Figure 15 pH-values on days 4 (n = 3 per group), 11 (n = 3 per group), 49 (n = 3 per group), and 90 (n = 5 per group in groups 1-5, n = 3 in group 6) of a silage trial testing 6 different silage additives (in house analysis). Groups 2 to 6 vary in proportions of *L. buchneri* and *L. plantarum* e.g. group 2 was inoculated with 94% *L. buchneri* and 6% *L. plantarum*

Table 1. Mean (standard deviation) of fermentation characteristics of grass silage on day 90 after ensiling including 6 treatment groups (1 = control group without additives, and groups 2 to 6 with varying proportions of *L. buchneri* / *L. plantarum*: 2 = 94% / 6%, 3 = 85% / 15%, 4 = 70% / 30%, 5 = 50% / 50%, 6 = 0% / 100%).

Fermentation characteristics		Control / Group 1 (n = 5)	Group 2 (n = 5)	Group 3 (n = 5)	Group 4 (n = 5)	Group 5 (n = 5)	Group 6 (n = 3)	P-Value
		mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	
Dry matter	g / kg FM	377 (4)	368 (6)	364 (12)	367* (10)	358* (3)	368 (14)	0.036
Weight loss	% FM	0.82 (0.02)	0.94* (0.04)	0.90* (0.03)	0.88* (0.02)	0.82 (0.01)	0.78* (0.01)	<0.001
pH		4.17 (0.019)	4.17 (0.029)	4.13* (0.009)	4.12* (0.019)	4.11* (0.004)	4.11* (0.017)	0.002
Lactic acid	% DM	9.99 (0.25)	10.04 (0.27)	10.02 (0.92)	9.96 (0.40)	10.21 (0.23)	10.07 (0.55)	0.8
Acetic acid	% DM	1.68 (0.02)	2.17* (0.09)	2.08* (0.20)	1.90* (0.11)	1.75 (0.05)	1.49* (0.11)	<0.001
1, 2-Propanediol	% DM	0.07 (0.01)	0.33* (0.04)	0.29* (0.06)	0.21* (0.03)	0.18* (0.03)	0.10* (0.01)	<0.001
Ethanol	% DM	0.41 (0.008)	0.41 (0.024)	0.40* (0.007)	0.39 (0.023)	0.35* (0.015)	0.35* (0.016)	0.001
ME	MJ/kg DM	11.0 (0.08)	11.0 (0.08)	11.0 (0.06)	11.0 (0.07)	10.9 (0.09)	10.9 (0.13)	0.5
NH3-N	% total N	5.34 (0.14)	5.81 (0.69)	5.88* (0.23)	5.70* (0.14)	6.05* (0.10)	5.40 (0.43)	0.02
Aerobic stability	d	12.6 (1.6)	16.5* (2.3)	15.5* (3.2)	11.3 (1.3)	10.0* (0.8)	10.4 (1.1)	0.002

* Significant difference from control group ($P < 0.05$).

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Chemical Composition of King Grass Silages with Inclusion of *Tithonia diversifolia*

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Keywords: Digestibility, forage conservation, protein, tree marigold.

Introduction

Tithonia diversifolia (TD) is a plant known for its benefits in environmental management, especially in the context of greenhouse gas (GHG) reduction. Studies indicate that the inclusion of TD can influence the chemical composition and digestion efficiency (Huertas-González et al., 2021). In this context, the combination of King Grass (KG - *Cenchrus purpureus*) and *Tithonia diversifolia* plants in the form of silage may be a viable alternative for ruminant diets. Therefore, the objective of this study was to evaluate the nutrient composition of King Grass silages with different TD inclusions.

Materials and methods

The experiment and in vitro analyses were carried out at the Colombian Agricultural Research Corporation (Agrosavia), located in Bogotá, Colombia. The plants (TD and King Grass) were harvested manually 60 days after regrowth. After harvest, the forage mass was spread in 15 cm high layers, without compaction, until reaching a dry matter content of $\pm 23\%$, totaling 24 h of drying. Immediately afterwards, they were processed in a stationary machine regulated to cut into a theoretical average particle size of 10 mm. Six treatments were evaluated: T1: FF KG – exclusive fresh king grass forage; T2: FF TD – exclusive fresh TD forage; T3: 100 KG - exclusive KG silage; T4: 70 KG 30 TD – 70% KG and 30% TD silage; T5: 50 TD: 50 KG- 50% TD and 50% KG silage and T6: 100 TD – Exclusive TD silage. Experimental 3-L silos (3.5 kg/silo) were used, sealed and kept at room temperature of approximately 25°C for 34 days. Fresh samples were collected at the time of ensiling ($n = 2$) and after opening the silos of each replicate ($n = 16$). The samples (before and after ensiling) were dried and ground in a Wiley mill with a 1.0 mm sieve. The samples were then sent to the laboratory for bromatological analysis of ash, crude protein (CP), neutral detergent fiber (NDF), non-fibrous carbohydrates (NFC), starch, ether extract (EE), DM digestibility (DMD), and also of secondary compounds, such as alkaloids, phenols, tannins, saponins, and sterols. The analyses were performed by near-infrared reflectance spectroscopy (NIRS) applying a calibration curve generated by Ariza-Nieto et al. (2018) from the Agrosavia® domain. A principal component analysis (PCA) was performed (using SAS PRINQUAL and SAS PROC PRINCOMP) to evaluate and characterize the chemical composition of the treatments.

Results

According to Figure 1, the 1st principal component (Component 1) explained 62% of the total data variance with an eigenvalue of 8.09, while the 2nd principal component (Component 2) accounted for 17% of the data variability with an eigenvalue of 2.22. Together, these two components explained 79% of the total data variation. The 1st component showed a strong association with nutritional quality parameters based on the eigenvectors, being influenced mainly by NDF (-0.35), DMD (0.34), NFC (0.32), starch (0.32), and CP (0.30). Thus, that axis differentiates silages with high inclusion of TD as having better nutritional composition (at right) than those with higher KG inclusion (at left). On the other hand, 2nd component was strongly influenced by secondary metabolites, with emphasis on tannins (-0.574), alkaloids (0.38), and saponins (0.38). According to Silva-Déley (2023), these compounds can impact digestibility and fermentation, which is evidenced by the strong association of fresh forages containing TD (FF TD) with tannins, and phenols. Fresh King Grass (FF KG) was grouped with NDF, saponins, and phenols, suggesting a higher structural fiber content, which may reduce forage digestibility and palatability. The biplot reinforces these associations, indicating that exclusive TD silages were positively correlated with higher CP, starch, NFC, and DMD, suggesting a nutritional advantage compared to silages with a lower proportion of TD. In contrast, fresh forages showed a greater correlation with tannins, saponins, and phenols, which may limit digestive efficiency. Intermediate inclusion levels (30% TD: 70% KG and 50% TD: 50% KG) were positioned centrally in the graph, suggesting a balance between the nutritional benefits of TD inclusion and the possible negative effects of secondary metabolites. This indicates that moderate levels of TD inclusion can optimize the nutritional value of silages without significantly compromising digestibility.

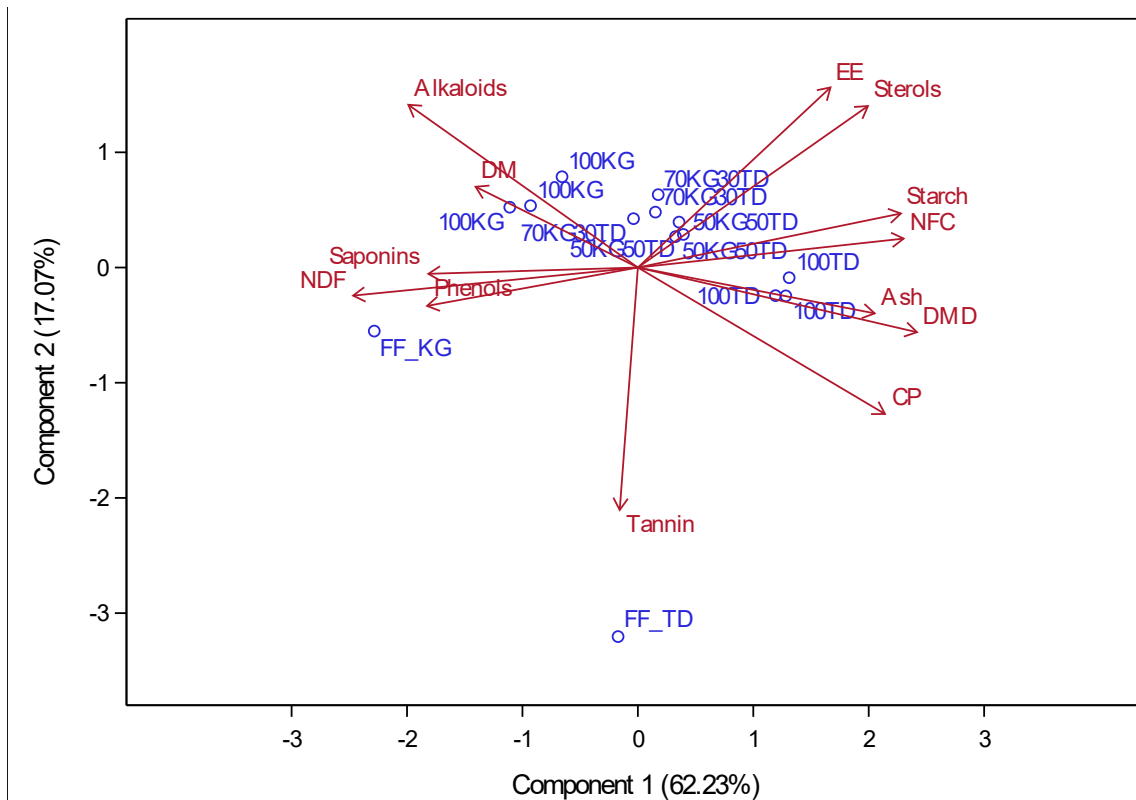


Figure 1. Biplot characterizing chemical composition of the six treatments (T1: FF KG – exclusive fresh king grass forage; T2: FF TD – exclusive fresh TD forage; T3: 100 KG - exclusive KG silage; T4: 70 KG 30 TD – 70% KG and 30% TD silage; T5: 50 TD: 50 KG- 50% TD and 50% KG silage and T6: 100 TD – Exclusive TD silage) based on the variables: alkaloids, crude protein (CP), dry matter (DM), dry matter digestibility (DMD), ether extract (EE), ash, neutral detergent fiber (NDF), non-fibrous carbohydrates (NFC), phenols, saponins, starch, and sterols.

Conclusions: Including *Tithonia diversifolia* in King Grass silages altered its chemical composition, increasing CP, NFC, and DMD. In contrast, fresh forages (TD and KG) show high levels of tannins and phenols, which can impact fermentation. Mixing these forages as silage showed a balance between nutritional value and secondary metabolites, indicating a viable alternative to optimize ruminant diets.

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