Optimizing Preference, Intake, and Performance of Silage Fed Ruminants

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High-quality silage should be void of undesirable compounds that could negatively affect animal performance (Kung Jr. et al., 2018)

Characterization of Silage Quality

- **Chemical, physical, microbial, and organoleptic components** (Mahanna and Chase, 2003; Grant and Ferraretto, 2018; Kung Jr. et al., 2018; Bandla et al., 2024)

- **Indication of…**
  - **Type of fermentation that occurred**
  - **Palatability (will an animal eat it?)**
  - **Animal performance**

Common (Routine Lab Analysis) Fermentation End Products (nonvolatile and volatile) in Various Silages

Item	Legume silage <30–35% DM	Legume silage 45–55% DM	Grass silage 25–35% DM	Corn silage 30–40% DM	High-moisture corn 70–75% DM
pH	4.3–4.5	4.7–5.0	4.3–4.7	3.7–4.0	4.0–4.5
Lactic acid, %	6–8	2–4	6–10	3–6	0.5–2.0
Acetic acid, %	2–3	0.5–2.0	1–3	1–3	<0.5
Propionic acid, %	<0.5	<0.1	<0.1	<0.1	<0.1
Butyric acid, %	<0.5	0	<0.5–1.0	0	0
Ethanol, %	0.5–1.0	0.5	0.5–1.0	1–3	0.2–2.0
NH_3-N, % of total N	10–15	<12	8–12	5–7	<10

Kung Jr. et al., 2018; J. Dairy Sci. 101: 4020-4033

Volatile Organic Compounds (VOC) in Silage (Bandla et al., 2024)

VOC	Major Producers/Pathway	Concentration, g/kg DM	Effect on Silage Quality
Acetic acid	heLAB, Enterobacterales	5 – 30	Aerobic stability
Butryic acid	Clostridia, Bacillus	<5	Indicator of reduced nutritive value
Propionic acid	LAB, Clostridia, Enterbacterales, PAB	<5	Aerobic stability?
Ethanol	Yeast, heLAB, Enterobacterales, Clostridia	5 – 30	High DM loss
2,3-butanediol	Enterobacterales, LAB, bacilli, yeast	<5	Indicator of reduced nutritive value
Propanol	heLAB, yeast, Clostridia	0.1 – 20	-
1,2-propanediol	heLAB, Clostridia	<2	Aerobic stability?
Esters	LAB, yeast, abiotic pathway	<0.5 – 5	Indicator of reduced nutritive value?
Iso-butyric acid	heLAB	1 – 2	-
Methanol	Crop maceration	0.3 – 3	-
Ketones	LAB, bacilli	0.1 – 2.0	-
Aldehydes	Crop maceration, abiotic pathway, LAB, yeast	~0.5	-

Desirable and Undesirable Volatile Organic Compounds (VOC)

- **Can influence silage preference and DMI via palatability and physiological effects (osmolality changes, rumen motility, hepatic oxidation, post-ingestive feedback)** (Bandla et al., 2024)

- **Many attempts to correlate the concentrations of silage acids, especially acetic acid and propionic acid, with effects on animal intake and performance…but results have been equivocal** (Mahanna and Chase, 2003; Huhtanen et al., 2007; Grant and Ferraretto, 2018; Bandla et al., 2024)

Amount and Source of Acetic Acid May Matter (Bandla et al., 2024)

- **Infusions of acetic acid, propionic acid, and butyric acid: reduced DMI**
  - Infused amount many fold higher than their presence in silage
  - Single vs blend of VOC in silage
    - Change in relative proportion of volatile metabolites (acids, alcohols, esters, or aldehydes) can influence silage preference and intake (Scherer et al., 2021)

- **Heterofermentative bacteria increase acetic acid in silage (use of an inoculant to improve feed-out stability)…the negative effect of acetic acid on intake is reduced or nonexistence**

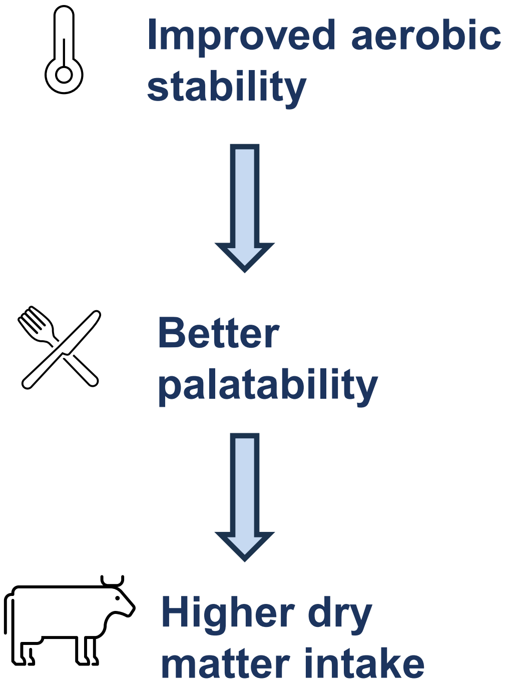
Palatability

- **Characterized based on chemical, microbial, or organoleptic factors** (Kung Jr. et al., 2018; Huhtanen et al., 2007)

- **Characterized based on ruminant feeding behavior/intake** (Gerlach et al., 2014)
  - **Greater or less intake of one feed rather than another**

- **Approaches can be used in tandem** (Spörndly and Åsberg, 2006; Miller-Cushon et al., 2014)

Can a multi-strain silage inoculant improve silage palatability, intake, and performance in dairy cattle?



Preference Trial – Haycrop Silage

- Aim: evaluate palatability of haycrop silage immediately and 48 hours after defacing through dry matter intake

Pape et al., 2024

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Small Ruminant Research
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Aerobic exposure of grass silages and its impact on dry matter intake and preference by goats

K. Gerlach [a], F. Roß [b], K. Weiß [c], W. Büscher [b], K.-H. Südekum [a]

Preference Trial – Haycrop Silage

- **Grass/alfalfa was harvested and ensiled**
- **Forage was either untreated (A) or treated with a multi-strain inoculant (B)**
  - Inoculant contained: *Pediococcus pentosaceus* (NCIMB 12455), *Lentilactobacillus buchneri* (NCIMB 40788), and *Lentilactobacillus hilgardii* (CNCM I-4785)
  - Dose: 2.5 $x10^5$ cfu/g fresh forage
- **Forage was packed in two experimental bunkers**
  - 19.8m x 6.1m x 2.0m
  - Bunkers were covered with oxygen barrier and 5mm UV stabilized film
- **Bunkers were opened after 55 days of ensiling for feed-out**
- **Untreated (A) and treated (B) silage at 0 and 48 h after defacing**

Adaptation Period

- 12 heifers exposed to all four treatments
  - **A** freshly defaced (0) – **A0**
  - **A** 48 h after defacing (48) - **A48**
  - **B** freshly defaced (0) – **B0**
  - **B** 48 h after defacing (48) – **B48**

A0

A48

B0

B48

30-minute offering

Ad-lib TMR

Ad-lib TMR

Adaptation period (4 days)

Treatment Period (Paired Comparison)

3-hour offering

Ad-lib TMR

30-minute DMI

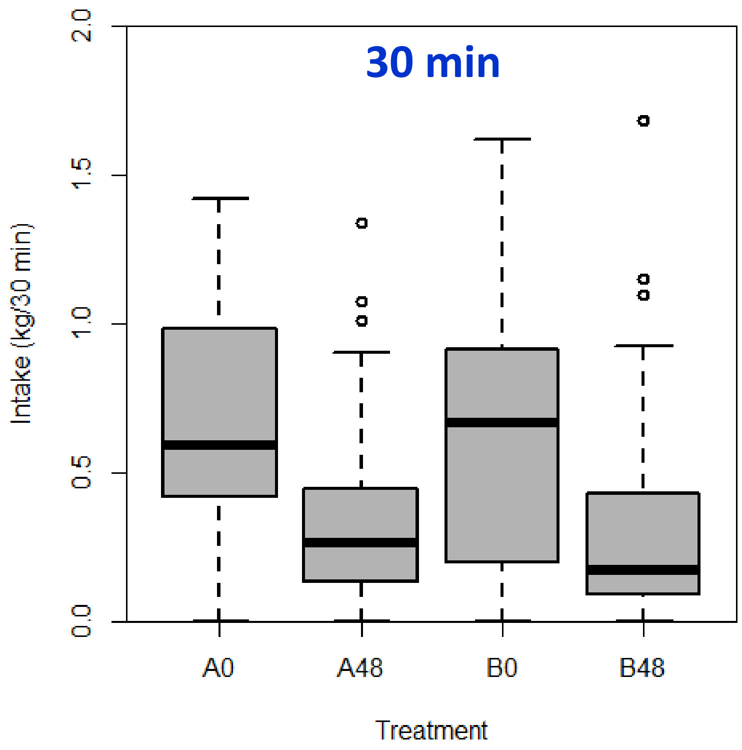
3-hour DMI

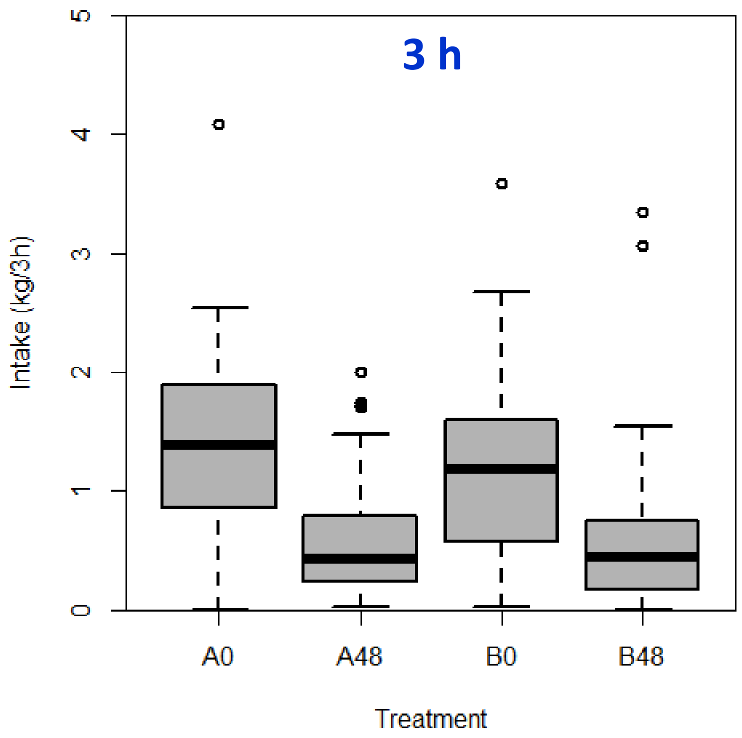
Ad-lib TMR

Experimental period (6 days)

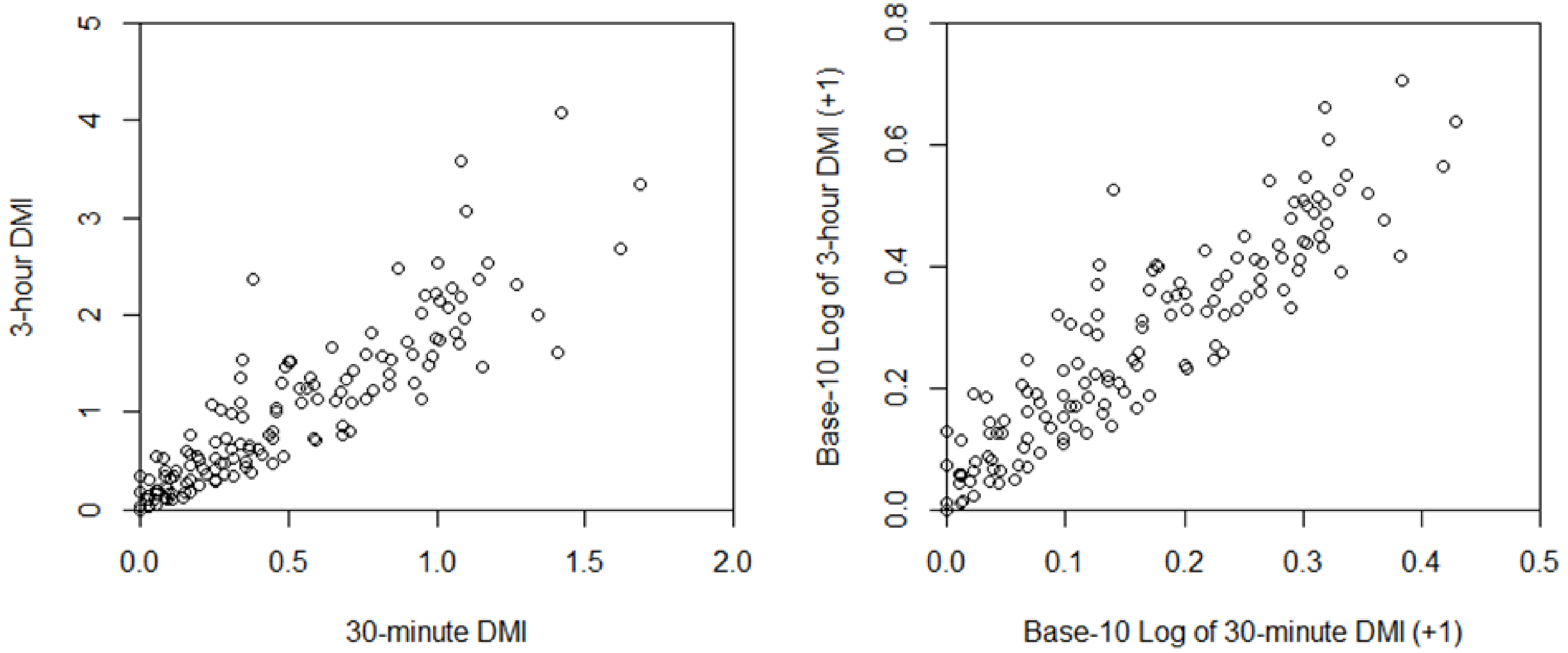
- **TMR removed from feed box 1 h before treatments offered**
- **10 kg of respective treatments offered**
- **After 30 min, forages weighed and returned to heifer**
- **Additional forages added if needed**
- **After 3 h, remaining forages weighed**
- **TMR fed ad libitum**
- **Each heifer received every combination of treatments with right/left orientation of treatments randomized across 6 d**

Silage intake: a simplistic overview





Relationship between 30 min and 3 h silage intake



Aerobic Exposure Affected Intake

DMI (kg)	Inoculant Treatment (IT) and Aerobic Exposure (AE)					*P*-values		
	A0	A48	B0	B48	SE	IT	AE	IT x AE
30 min	0.65	0.36	0.59	0.33	0.07	0.49	<0.01	0.76
3 h	1.41	0.62	1.24	0.66	0.15	0.55	<0.01	0.36

Effect of silage treatments on intake were analyzed using the MIXED procedure in SAS v. 9.4

0 h vs 48 h

30 min - 0.62 kg vs 0.35 kg

3 h – 1.32 kg vs 0.64 kg

Statistical Analysis – Preference Testing

- **Paired Comparison**
  - Initially proposed Multi-dimensional scaling (MDS)
    - However, MDS measures degree of difference, but not direction of difference…not best approach
  - Bradley-Terry model used – well-established method for modeling paired comparison data in animal and human behavior research

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Technical Note
Health, Welfare, and Behavior

Multiverse analysis and the Bradley-Terry model: A proposed approach for evaluating palatability and preference

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Abstract: The palatability of feed for dairy cows is an important consideration but is difficult to measure, particularly when considering more than 2 feeds. We outline how a combination of multiverse analysis and Bradley-Terry modeling, 2 methodological tools that have rarely been applied in dairy science, can be adapted to address this problem. Specifically, we propose to apply multiverse analysis as a way to consider a range of thresholds for how much of a mixed grass-legume (MGL) silage had to be consumed (as a percent of the total DMI) to be designated as preferred. Each threshold gives rise to a separate dataset and a corresponding fitted Bradley-Terry model. Bradley-Terry models attribute to each feed what is commonly referred to as an "ability" in the context of sports or other competitions but can be interpreted as palatability when applied to feeds. This combined approach is a way of estimating palatabilities that appropriately reflect the degree of preference cows express through their feeding behavior. It has the advantages of being transparent and relatively easy to implement. A possible disadvantage is that this method is limited to a paired comparison approach and has difficulties with main-effects statistical inference. We demonstrate the use of this methodology on an example dataset comparing MGL silages under different ensiling conditions and exposed to oxygen for different durations.

Two-step Analysis for Analyzing Preference Based on DMI

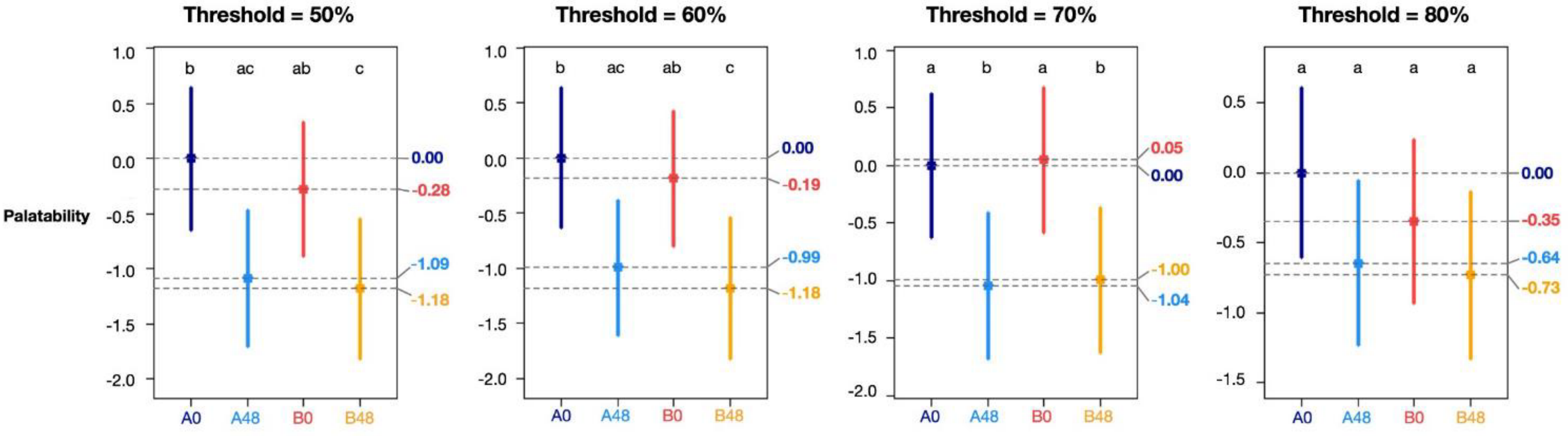
- *Multiverse analysis*: "tests a hypothesis on multiple versions of a dataset, and considers the results collectively"
  - Thresholds: 50%, 60%, 70%, 80%

- *Bradley-Terry method*:

$$\Pr(i \text{ defeats } j) = \frac{1}{1 + e^{\lambda_j - \lambda_i}}$$

  - "Paired comparisons approach"
  - Sport analogy: "attributes an 'ability' to the entities in competition" → probability of any given team will defeat another

Estimates and 95% CI of Palatability by Treatment for Selected Win-Loss Thresholds Applied to 30-min Intake Data



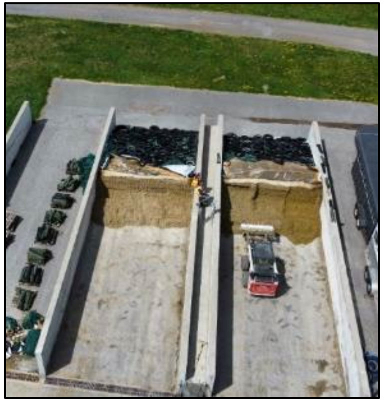
A0 was designated as the reference level so was assigned a palatability of zero

Pape et al., 2024

Preference Trial – Corn Silage

- **Conventional corn hybrid was harvested and ensiled**
- **Forage was either untreated (control; C) or treated with a multi-strain inoculant (treated, T)**
  - Inoculant contained: *Pediococcus pentosaceus* (NCIMB 12455), *Lentilactobacillus buchneri* (NCIMB 40788), and *Lentilactobacillus hilgardii* (CNCM I-4785)
  - Dose: 2.5 x10^5 cfu/g fresh forage
- **Forage was packed in two experimental bunkers**
  - 19.8m x 6.1m x 2.0m
  - Bunkers were covered with oxygen barrier and 5mm UV stabilized film
- **Bunkers were opened after 189 days of ensiling for feed-out**

Ballard et al., 2025



Inoculated silage had reduced yeasts counts

Sampling Time	Treatment	Total lactic acid bacteria[1]		Total yeasts[1]		Total Molds[1]	
		50 cm	150 cm	50 cm	150 cm	50 cm	150 cm
Day 0	Control	7.58	7.63	5.12	3.15	1.50	1.30
	Treated	7.37	7.59	2.87	2.08	1.50	1.50
	P-value	0.117	0.754	**0.009**	0.175	NA	0.317

[1]Expressed as log-transformed colony-forming units per gram of fresh material; limit of detection is <2.00 CFU/ g
NA; not applicable.

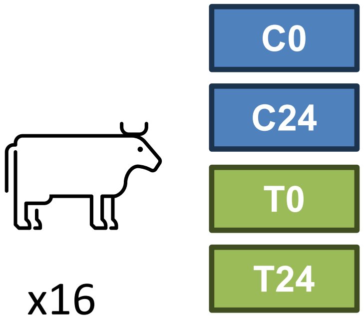
Inoculated silage had slight improvement in aerobic stability

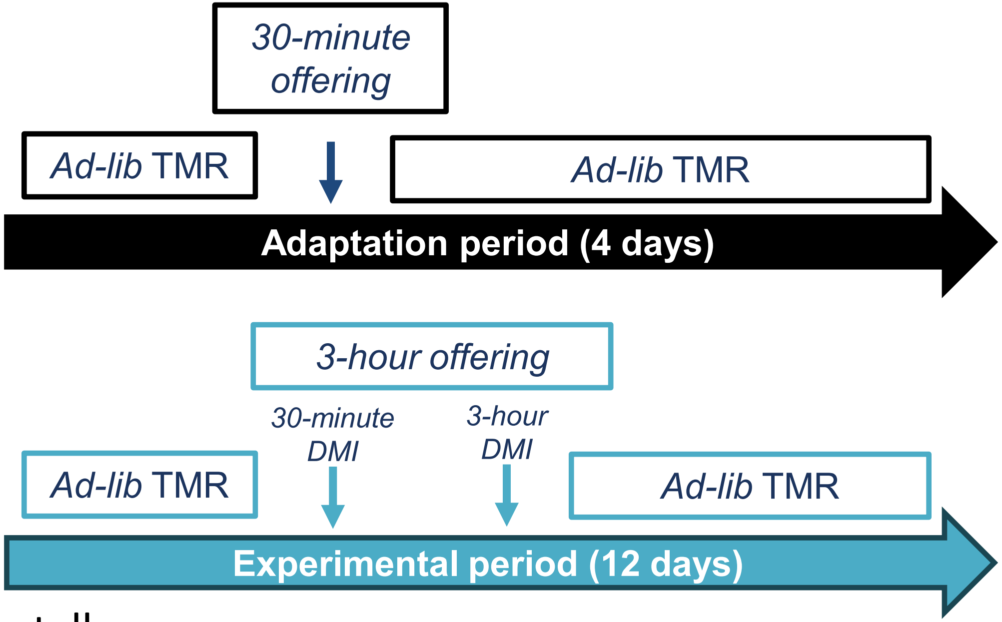
Sampling Time	Treatment	Time spent above 2°C[1]		Maximum temperature (°C)[2]		AS Deterioration coefficient[3]	
		50 cm	150 cm	50 cm	150 cm	50 cm	150 cm
Day 0	Control	196.15	146.97	37.25	3.15	3.33	3.12
	Treated	153.48	148.03	33.74	2.08	3.01	3.06
	P-value	**0.028**	0.917	0.175	0.917	**0.009**	0.465

[1]Hours spent above the temperature threshold over ambient temperature
[2]Maximum temperature reached during 10-day aerobic stability assay
[3]Log-transformed summation of temperatures above the 2°C threshold above ambient temperature

Ballard et al., 2025

Preference Trial – Corn Silage





- 16 heifers were housed in tie-stalls
- Untreated (C) and treated (T) corn silage at 0 and 24 h after defacing
- TMR was removed from feed bunk 1 hour before feeding
- Left/right orientation of treatments were randomized

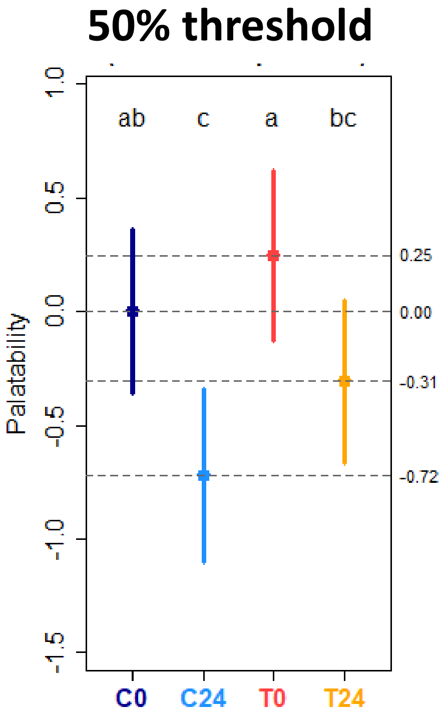
Ballard et al., 2025

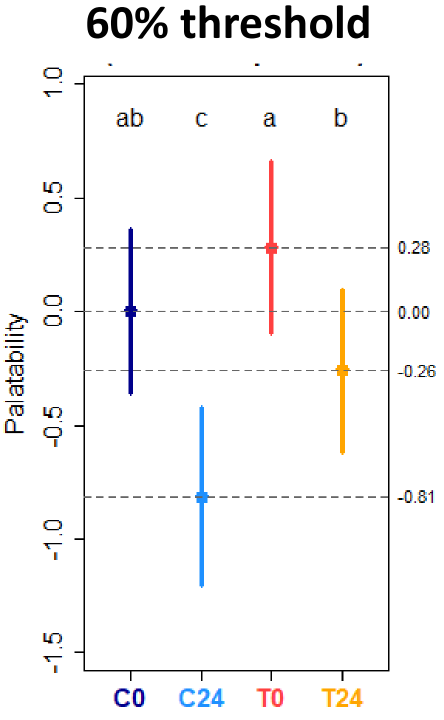
Untreated Silage and Aerobically Exposed Silage had Reduced DMI

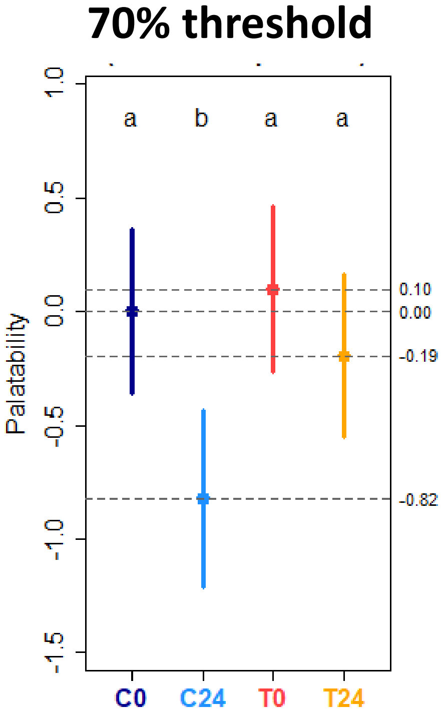
	Inoculant Treatment (IT) and Aerobic Exposure (AE)					*P*-values		
DMI, kg	C0	C24	T0	T24	SE	IT	AE	IT x AE
30 min	1.13	0.75	1.22	1.07	0.09	0.01	<0.01	0.16
3 h	2.86^{a}	2.10^{b}	2.72^{a}	2.53^{ab}	0.18	0.39	<0.01	0.08

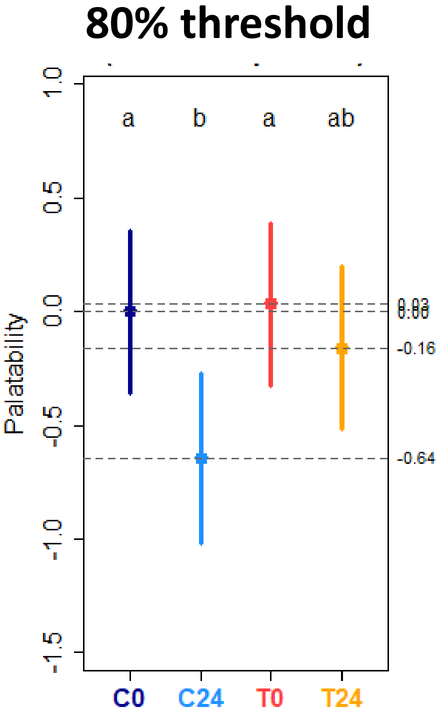
^{a,b} Differing superscripts are significantly different ($P \leq 0.05$)

Heifers preferred treated-silage over untreated-silage when offered aerobically exposed silage (30-min intake)



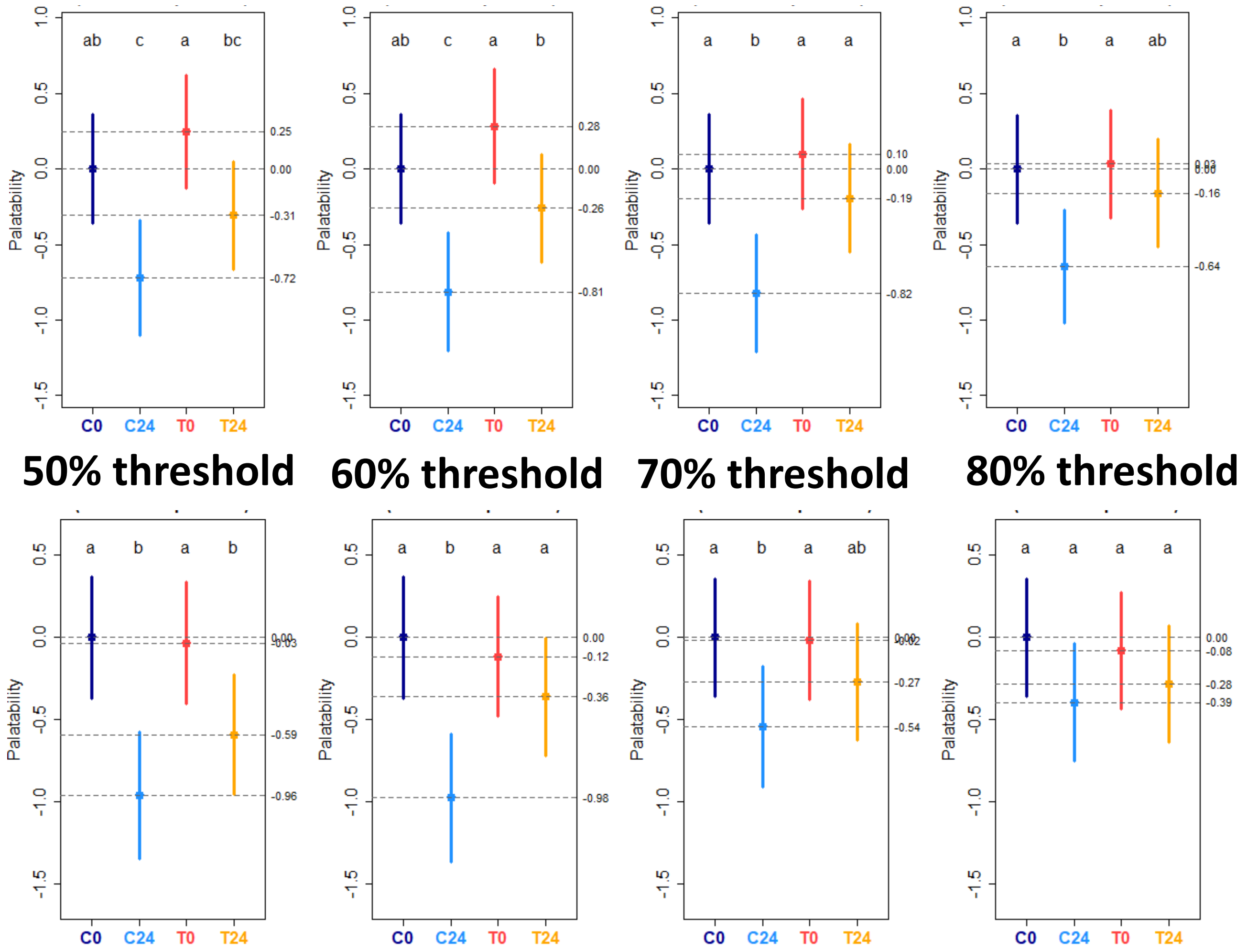






Heifers preferred treated-silage over untreated-silage when offered aerobically exposed silage

30- min



3-h

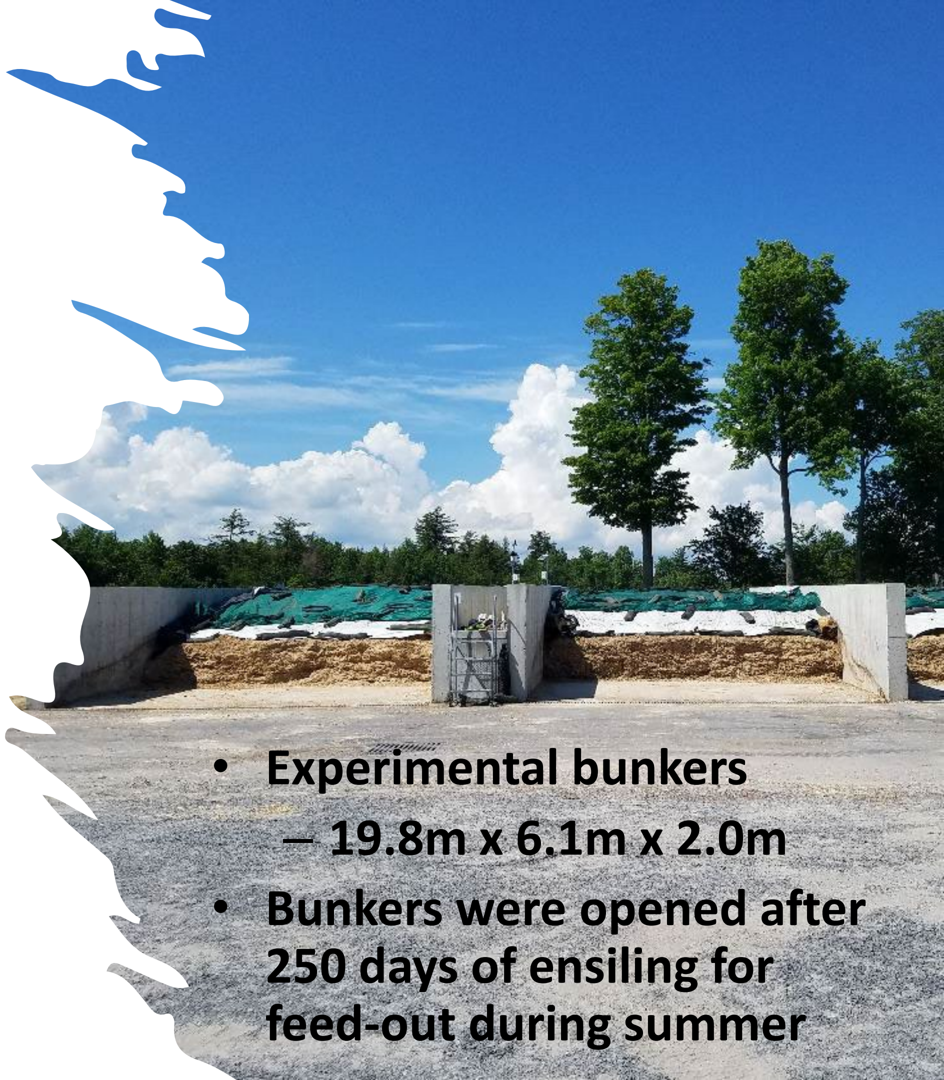
Evaluation of a Multi-strain Silage Inoculant on Intake and Lactation Performance of Cows

- **Randomized block design with repeated measurements**
  - **46 Holstein cows**
    - **Fed 1x/d, milked 3x/d**
  - **2 wk covariate period**
  - **9 wk treatment period**

Dann et al., 2025

Harvest and Ensiling of Whole Plant Corn

- Corn was either untreated (CON) or treated with a multi-strain inoculant (MSI)
  - Inoculant contained: *Pediococcus pentosaceus* (NCIMB 12455), *Lentilactobacillus buchneri* (NCIMB 40788), and *Lentilactobacillus hilgardii* (CNCM I-4785)
  - Dose: 2.5 x10^5 cfu/g fresh forage



- Experimental bunkers
  - 19.8m x 6.1m x 2.0m
- Bunkers were opened after 250 days of ensiling for feed-out during summer

Composition of corn silage that was not (CON) or was inoculated with a multi-strain inoculant (MSI) at harvest

Item	CON Corn Silage	MSI Corn Silage
Dry matter, % of as-fed	36.6±1.7[1]	40.0±0.9
Crude protein, %	6.6±0.4	6.7±0.2
Starch, %	38.8±1.4	39.0±0.8
Neutral detergent fiber (NDF), %	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, %	**4.6±0.2**	**1.5±0.2**
Acetic acid, %	**1.7±0.3**	**3.5±0.5**
pH	3.9±0.0	4.2±0.0

[1] Mean ± standard deviation

Ingredient and chemical composition of diets

Item (DM basis)	CON Diet	MSI Diet
Ingredient		
CON corn silage, %	**50.00**	-
MSI corn silage, %	-	**50.00**
Haycrop silage, %	6.67	6.67
Corn and soy-based grain mix	43.33	43.33
Composition		
Dry matter, % of as-fed	49.4± 1.4[1]	54.4± 1.3
Crude protein, %	15.9±0.7	16.0±0.6
Neutral detergent fiber, %	29.0±1.0	29.8±0.8
Starch, %	28.4±1.4	28.5±1.1
Sugar (ESC), %	3.8±0.3	3.8±0.3
Fat (ether extract), %	3.0±0.3	2.8±0.1

[1] Mean ± standard deviation

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

				P-value		
	CON Diet	MSI Diet	SE	TRT	Time	TRT x Time
DMI, kg/d	**25.7**	**25.4**	0.2	0.34	<0.001	0.19
DMI, % of BW	3.74	3.70	0.03	0.24	<0.001	0.47
BW, kg	690	688	3	0.53	<0.001	0.58
Milk, kg/d	46.8	46.9	0.4	0.77	<0.001	0.91
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
Fat, %	3.54	3.51	0.07	0.74	<0.001	0.29
True protein, kg/d	1.35	1.35	0.01	0.99	<0.001	0.61
Ture protein, %	2.90	2.89	0.01	0.64	<0.001	0.23
ECM/DMI	**1.81**	**1.85**	0.02	0.14	<0.001	0.11

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

Item	CON Corn Silage	MSI Corn Silage
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
Spoilage/not of quality to feed, % of material removed	28.6	16.4

Case scenario for using silage inoculants for 100 as-fed metric tons of chopped whole plant corn

Item	CON Corn Silage	MSI Corn Silage
Material ensiled, kg as-fed	100,000	100,000
Fermentation loss, kg as-fed (%)	4,100 (4.1)	3,400 (3.4)
Material available after fermentation loss, kg as-fed	95,900	96,600
Material available after fermentation loss, kg DM	35,099	38,640
Spoilage, %	28.6	16.4
Material available to feed (good quality), kg DM	25,061	32,303
ECM produced, kg	**91,663**	**119,292**
ECM produced, kg/material ensiled, kg DM ensiled	**2.50**	**2.98**

Key Findings

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

Take Home Messages

- Some VOC may affect intake, metabolism, and performance of ruminants because of the VOC chemical structure and sensory characteristics

- Use of preference studies based on feeding behavior and intake may help elucidate which type or quality of silage will affect intake and ultimately performance

- By understanding the factors that influence silage preference, silage ensiling and feed-out management practices can be improved

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