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Manipulation of Silage Fermentation for Animal Health

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July 21, 2025

Background

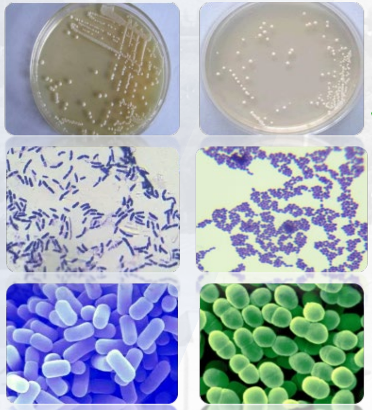
Importance of silage to animal husbandry

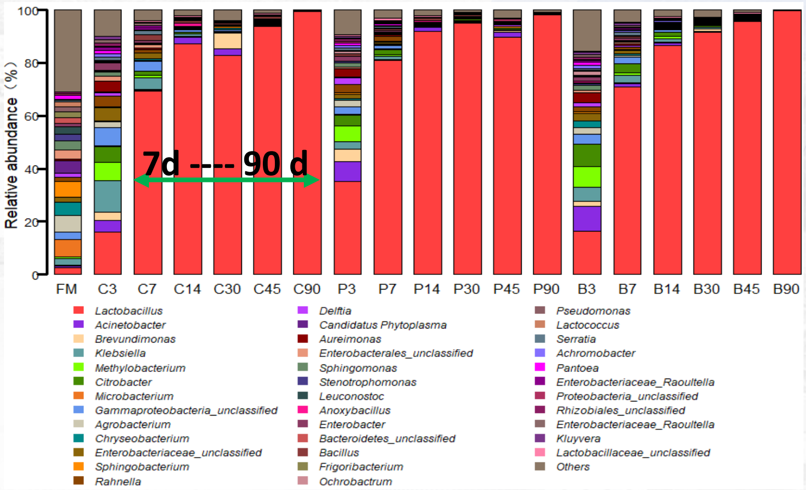
- Silage accounts for more than 50% of dairy cow ration.
- There are about 240 million dairy cattle, 1 billion beef cattle worldwide (FAO, 2022).
- Consuming about 7.4 billion tons of silage per year.

Background

Importance of lactic acid bacteria to silage

- Lactic acid bacteria (LAB) is the major fermenter of ensiled forages;
- After 7 d fermentation, LAB accounts for 68% of total bacteria in naturally fermented corn silage;
- After 90 d fermentation, LAB accounts for 98% of total bacteria in corn silage.





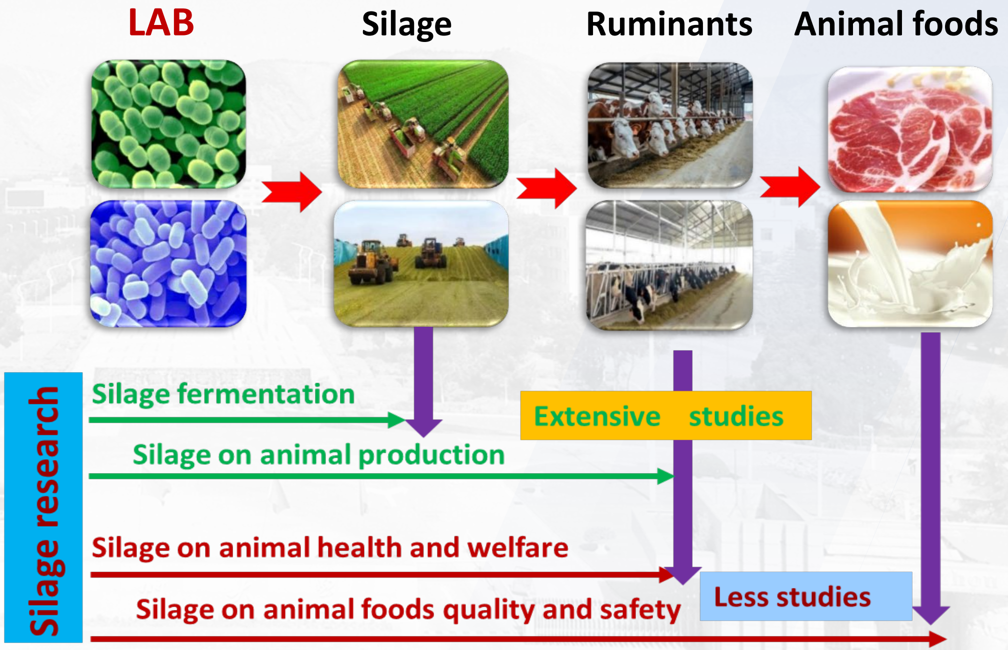
Xu et al., *Microbial Biotechnology*, 2021.

Background

Importance of inoculating LAB at ensiling to animal production system

Advances of Silage Research

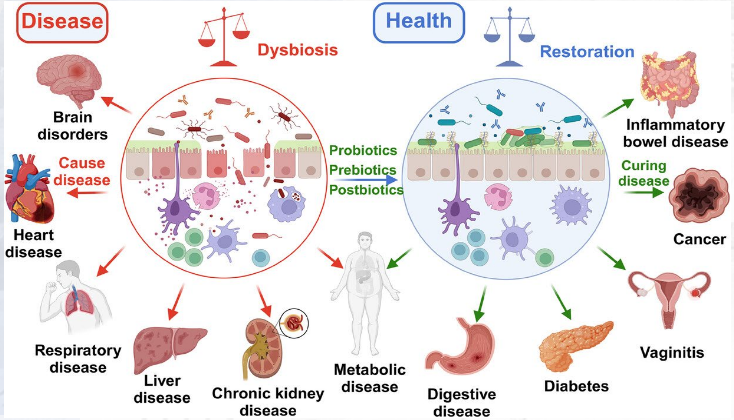
- Inoculants on silage fermentation
- Inoculants on animal production
- **Inoculants on animal health and welfare!**
- **Inoculants on animal foods quality and safety!**



Background

Importance of lactic acid bacteria to health

- LAB is not only a **fermenter** but also one of the important **probiotics**
- LAB is beneficial to human health and has been widely used in clinical diseases



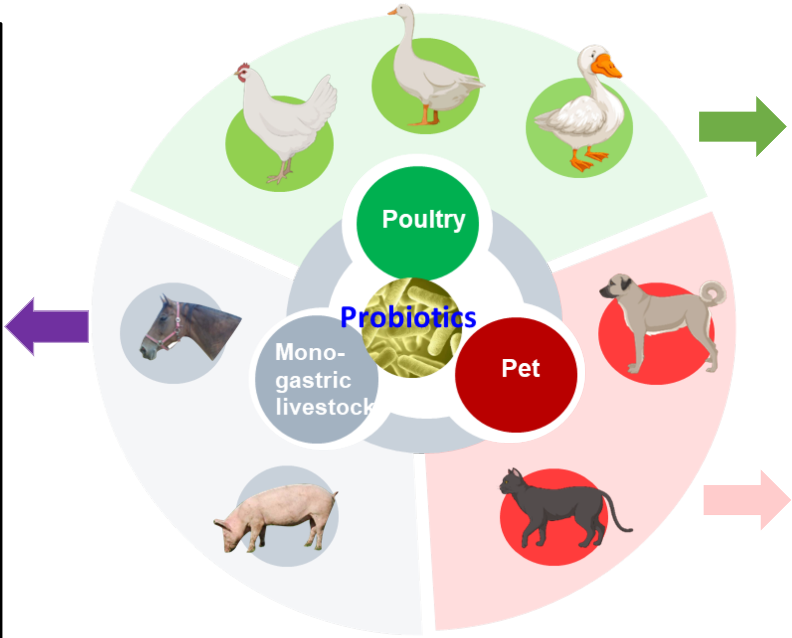
Tremendous studies have confirmed that LAB can be used to ameliorate clinical diseases via restoration of gut microbiota (Jenny Tschiesche, Gut health and Probiotics, 2018).

Background

Direct-fed probiotics for improving mono-gastric animal health

Probiotics:
E. faecalis
E. faecium
Bacillus cereus
B. subtilis
B. licheniformis
L. reuteri
L. Acidophilus

Effects to animal health:
- Mortality reduction
- Increase immunity
- Improve gut health
- Reduction of diarrhea
- Decrease in stress
- Avoid hindgut disorders



Probiotics:
L. animalis *L. fermentum*
L. salivarius *L. acidophilus*
S. faecium *L. reuteri*
E. faecium *S. cerevisiae*

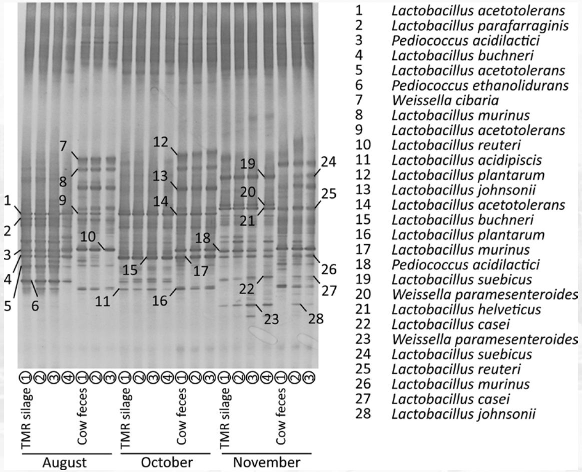
Effects to animal health:
- Mortality reduction
- Increase immune response
- Improve gut health

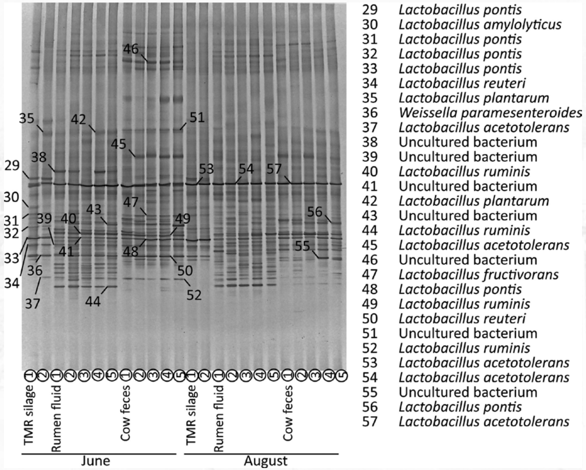
Probiotics:
E. hirae *E. faecium*
L. reuteri *L. rhamnosus*
L. plantarum *W. cibaria*
L. acidophilus *B. subtilis*

Effects to animal health:
- Clinical recovery
- Increase immune function
- Improve gut health
- Decrease in diarrhea rate

Background

- Unlike mono-gastric animals, ruminants consuming silage containing plenty lactic acid bacteria everyday!
- Silage can be an vehicle to deliver probiotics to the hindgut of ruminants.





A total of 14 LAB species were detected in the TMR silage samples, of which 5 (*L. acetotolerans*, *L. pontis*, *L. casei*, *L. suebicus*, and *L. plantarum*) were detected in the dairy cow feces.

Although the gut LAB community is robust and not easily affected by the silage conditions, several LAB species can inhabit both silage and feces, which suggests the potential of using silage as a vehicle for conveying probiotics (Han H, et al. J Dairy Sci. 2014).

Background

Contaminants in silage endangering animal health

Mycotoxins

- Pathogenic secondary metabolites mainly biosynthesized by filamentous fungi.

Pesticides

- Toxic substance used to kill pest and control plant disease that cause damage to crop.

1
Feed intake
Milk yield
Reproductive system
Immunosuppression

2
Neurological disorder
Cancer
Birth defect
Endocrine system

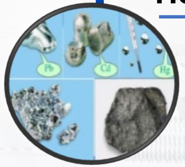
3
Neurological disorder
Cancer
Birth defect
Endocrine system

4
Reproduction
Immunity
Feed intake
Antioxidant system

Antibiotic resistance genes (ARGs)

- Antibiotics created microbial ARG selection pressures, inducing ARG emergence and spreading.

Heavy metals and other toxins



- Heavy metals: Cr, Pb, Cd, As, Hg.
- Other toxic compounds

Manipulation of silage fermentation: Prospects and opportunities

There are great opportunities to:

- Develop novel LAB inoculants to manipulate silage fermentation for making functional or probiotic silage with health beneficial properties
- Develop novel LAB inoculants to manipulate silage fermentation for mitigating hazardous substances in silage

LAB isolation

Silage making

Animal feeding

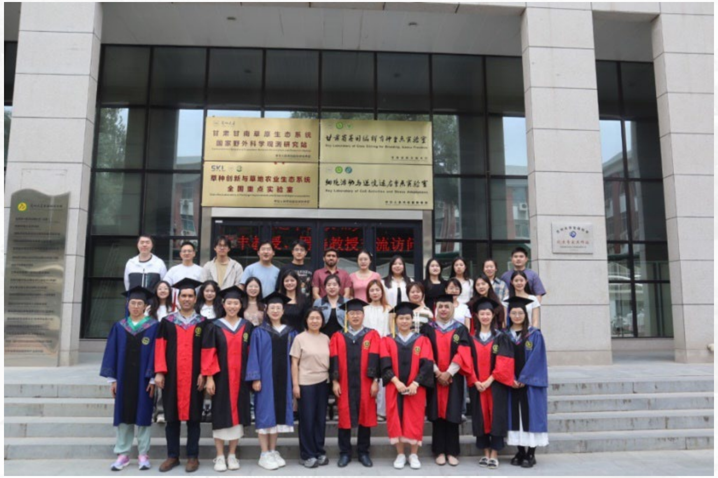
To improve:

- **Animal production**
- **Animal food quality and safety**
- **Animal health**

Manipulation of silage fermentation by specific LAB strains to produce functional silage for animal health

Current research work from our group:

- Probiotic-derived antioxidative silage
- Probiotic-derived anti-inflammatory silage



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Probiotic-derived antioxidative silage for animal health

- Antioxidants additives have been widely used in animal production
- Certain LAB strains exhibit robust redox regulatory capabilities, have high antioxidant activities

Application of antioxidant-rich LAB to improve silage antioxidant activity

Animal Feed Science and Technology 268 (2020) 114614

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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The effect of *Pediococcus acidilactici* J17 with high-antioxidant activity on antioxidant, α-tocopherol, β-carotene, fatty acids, and fermentation profiles of alfalfa silage ensiled at two different dry matter contents

Y.X. Zhang[a,b], W.C. Ke[a,b], J. Bai[b,c], F.H. Li[b,c], D.M. Xu[a,b], Z.T. Ding[a,b,*], X.S. Guo[a,b,*]

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Zhang et al., *Anim Feed Sci Technol.*, 2020.

Animal Feed Science and Technology 272 (2021) 114751

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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ELSEVIER

Antioxidant status, chemical composition and fermentation profile of alfalfa silage ensiled at two dry matter contents with a novel *Lactobacillus plantarum* strain with high-antioxidant activity

Y.X. Zhang[a,1], W.C. Ke[a], D. Vyas[b], A.T. Adesogan[b], M. Franco[c], F.H. Li[a], J. Bai[a], X. S. Guo[a,*]

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Zhang et al., *Anim Feed Sci Technol.*, 2021.

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L. plantarum 24-7 with high-antioxidant activity

Ensiling trials

Feeding trial

Items	Lp. 24-7	Lp. BX62	Reference Strain
Survival rate at 2 mmol/L H_2O_2	1.53[a]	1.50[b]	0.148[c]
Fermentation supernatant antioxidant activity			
DPPH, %	87.12[a]	87.76[a]	69.14[b]
·OH, %	62.90[a]	61.86[b]	41.26[c]
O_2^-, %	25.40[b]	27.70[a]	11.64[c]
T-AOC, U/mL	31.08[a]	31.63[a]	24.91[b]
SOD, U/mL	65.49[a]	66.67[a]	47.10[b]
GSH-Px, U/mL	ND	ND	ND
CAT, U/mL	ND	ND	ND

Zhang et al.
Journal of Animal Science and Biotechnology (2024) 15:9
https://doi.org/10.1186/s40104-023-00977-3
Journal of Animal Science and Biotechnology

RESEARCH Open Access

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Effects of antioxidant-rich *Lactiplantibacillus plantarum* inoculated alfalfa silage on rumen fermentation, antioxidant and immunity status, and mammary gland gene expression in dairy goats

Yixin Zhang[1,2,3], Samaila Usman[1,2,3], Qiang Li[1,2,3], Fuhou Li[2,3], Xia Zhang[2,3], Luiz Gustavo Nussio[4] and Xusheng Guo[1,2,3*]

Zhang et al. *J Anim Sci Biotechnol.* 2024.

Table 1. Fermentation characteristic and antioxidant capacity of ensiled alfalfa

Item	Ttreatment		SEM	*P*-value
	Reference Strain	LP. 24-7		
Fermentation characteristic				
pH	4.62	4.63	0.008	0.832
Lactic acid, g/kg DM	42.3	43.2	0.33	0.189
Acetic acid, g/kg DM	17.6	18.5	0.13	0.005
Propionic acid, g/kg DM	5.38	4.66	0.091	0.003
Antioxidant capacity				
α-tocopherol, mg/kg DM	19.9	**26.3**	0.76	0.002
β-carotene, mg/kg DM	159	**209**	5.3	0.001
T-AOC, mmol/kg FW	7.58	**9.26**	0.093	< 0.001
SOD, U/g FW	785	**659**	11.7	< 0.001
GSH-Px, U/g FW	438	**488**	5.1	0.001
CAT, U/g FW	0.00	**9.80**	0.218	< 0.001

Note： T-AOC, total antioxidant capacity; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase.

Table 2. Effect of diets on milk production and quality in dairy goats

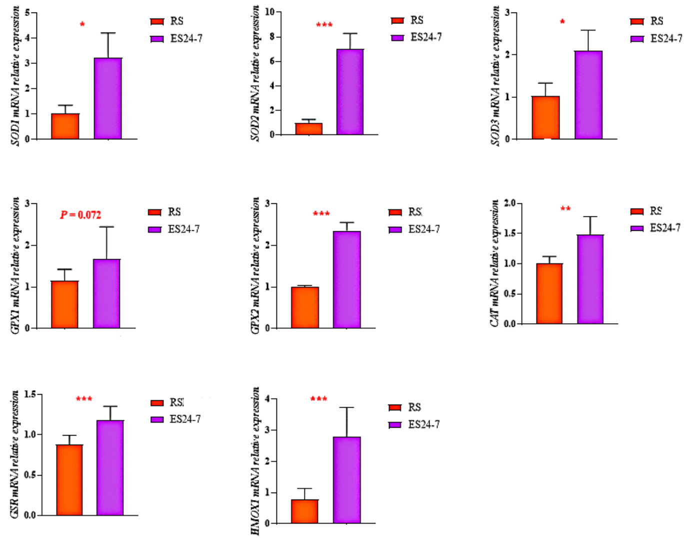
Items	Dietary treatment		SEM	*P*-value
	Reference strain	Lp. 24-7		
DMI, kg/d	0.811	0.778	0.043	0.717
Milk production				
Milk yield, kg/d	1.41	1.39	0.048	0.900
3.5% FCM, kg/d	1.36	1.42	0.014	0.058
3.5% FCM/DMI	1.67	1.83	0.024	0.006
Fat, g/d	46.4	50.3	0.85	0.040
Casein, g/d	39.7	47.3	1.31	0.011
Milk components				
Fat, %	3.30	3.60	0.061	0.024
Protein, %	3.76	4.14	0.113	0.115
Lactose, %	4.48	4.41	0.046	0.441
Casein, %	2.83	3.39	0.093	0.008
TS, %	11.5	12.3	0.17	0.035
SNF, %	8.97	9.62	0.139	0.035
Vitamin A, ug/100g	597	712	11.1	< 0.001

Table 3. Whey and serum antioxidant capacities of dairy goats

Items	Dietary treatment		SEM	*P*-value
	Reference strain	Lp. 24-7		
Whey antioxidant				
T-AOC, mM	0.087	0.091	0.006	0.786
SOD, U/mL	14.4	23.9	0.36	< 0.001
GSH-Px, U/mL	0.00	22.6	0.54	< 0.001
CAT, U/mL	0.131	0.602	0.052	0.005
Serum antioxidant				
T-AOC, mM	0.21	0.30	0.007	< 0.001
SOD, U/mL	62.7	95.2	1.73	< 0.001
GSH-Px, U/mL	102	190	2.5	< 0.001
CAT, U/mL	3.47	7.53	0.215	< 0.001

Note： T-AOC, total antioxidant capacity; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase.

Fig. 1 Relative expression of antioxidant-related genes in the **mammary gland** sampled from dairy goats

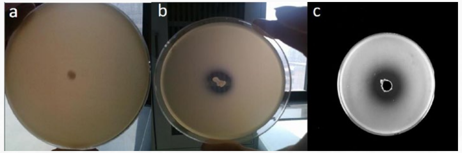


Feeding dairy goats with antioxidant-rich *L. plantarum* inoculated alfalfa silage:

1) increased the antioxidant status in milk and serum

2) Increased the expression of antioxidant-related genes in the mammary gland

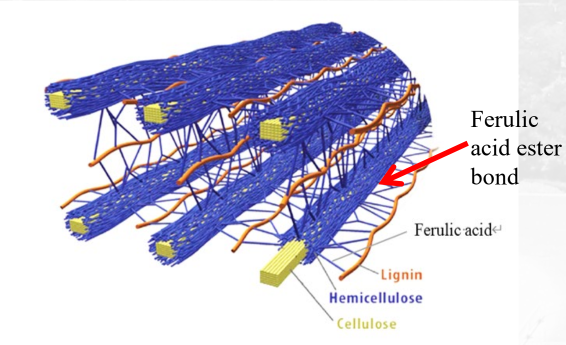
Application of ferulic acid esterase-producing LAB to improve silage antioxidant activity



Ferulic acid esterase-producing strain *L. plantarum* A1 (Lp A1)

Double-win of feruloyl esterase LAB in silage:
- Increase fiber digestibility
- Release ferulic acid into silage

Ferulic acid has strong antioxidant activity



Lignocellulose structure

Fig. 1. *In vitro* DM degradability of alfalfa silage

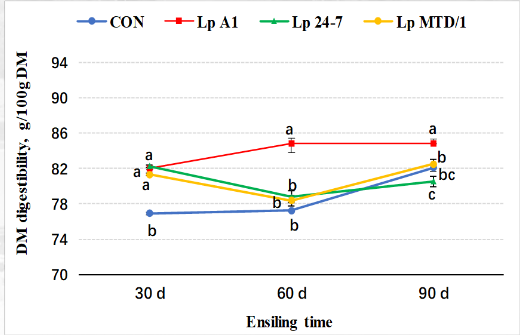
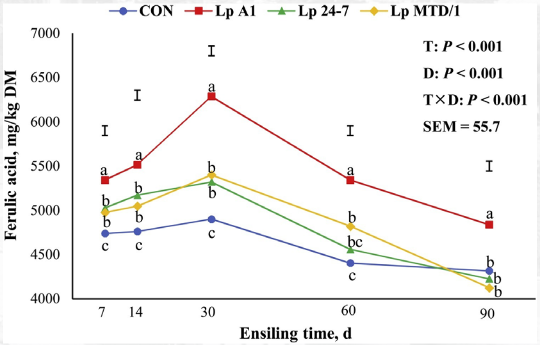


Fig. 2. Ferulic acid concentration in alfalfa silage



Ensiling trial

Feeding trial

Table 1. Chemical composition, ensiling characteristics and antioxidase activity of alfalfa silage (90 d)

Items	Silage treatment		SEM	*P*-value
	Control	Lp A1		
Chemical composition				
DM, g/kg FW	450	452	0.613	0.093
CP, g/kg DM	167	169	1.845	0.426
aNDF, g/kg DM	453	430	6.653	0.078
ADF, g/kg DM	345	328	5.407	0.135
Ferulic acid, g/kg DM	4.61	5.19	0.094	< 0.001
Fermentative characteristics				
pH	4.67	4.54	0.021	< 0.001
Lactic acid, g/kg DM	70.5	88.2	3.207	0.001
Antioxidase activity				
T-AOC, U/g FM	158	181	5.626	0.035
SOD, U/g FM	617	636	4.884	0.048
GSH-Px, U/g FM	788	839	9.236	0.002

Table 2. Intake and apparent digestibility of dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* A1

Items	Treatment diet		SEM	*P*-value
	Control	Lp A1		
Total intake, g/d				
DM	1370	1381	4.059	0.213
OM	1263	1271	3.707	0.285
CP	247	250	1.837	0.396
aNDF	467	452	2.175	< 0.001
ADF	310	298	1.615	< 0.001
Apparent digestibility, %				
DM	60.1	**63.7**	0.486	< 0.001
OM	62.3	**65.6**	0.459	< 0.001
CP	70.3	72.9	0.56	0.013
aNDF	37.8	39.0	1.034	0.582
ADF	32.3	32.5	1.105	0.921

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Table 3. Ruminal fermentation parameters in dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* A1

Items	Treatment diet		SEM	*P*-value
	Control	Lp A1		
pH	6.42	6.36	0.025	0.329
VFA concentration, mM				
Total VFA	**45.1**	**63.8**	2.828	< 0.001
Acetate	31.4	46.4	2.249	< 0.001
Propionate	6.14	8.76	0.436	0.001
Butyrate	5.19	5.89	0.358	0.342
Valerate	0.39	0.47	0.025	0.146
Isobutyrate	0.83	0.97	0.035	0.048
Isovalerate	1.13	1.30	0.050	0.103
Total BCVFA	1.97	2.27	0.083	0.070
VFA composition, % M				
Acetate	69.7	72.6	0.637	0.021
Propionate	13.5	13.8	0.442	0.719
Butyrate	11.5	9.22	0.528	0.029
Acetate/Propionate	5.27	5.34	0.185	0.850

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KeAi CHINESE ROOTS GLOBAL IMPACT

Original Research Article

Probiotic effect of ferulic acid esterase-producing *Lactobacillus plantarum* inoculated alfalfa silage on digestion, antioxidant, and immunity status of lactating dairy goats

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Fuhou Li [a, b], Baibing Zhang [a, b], Yixin Zhang [a, b], Xia Zhang [a, b], Samaila Usman [a], Zitong Ding [a, b], Lizhuang Hao [c], Xusheng Guo [a, b, *]

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Li et al. *Anim Nutrition.*, 2022.

Table 4. Serum and whey antioxidase activity from dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* A1

Items	Treatment diet		SEM	*P*-value
	Control	Lp A1		
Serum, U/mL				
T-AOC	11.6	13.7	0.362	0.040
SOD	96.3	120	8.323	0.007
GSH-Px	723	791	12.18	< 0.001
CAT	4.14	5.03	0.496	0.032
Whey, U/mL				
T-AOC	6.80	7.98	0.645	0.443
SOD	107	118	7.437	0.386
GSH-Px	167	214	9.603	0.021
CAT	4.31	4.70	0.322	0.275

Note: T-AOC, total antioxidant capacity; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase.

Table 5. Milk yield and milk composition of dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* A1

Items	Treatment		SEM	*P*-value
	Control	Lp A1		
Yields, g/d				
Milk, kg/d	0.74	0.78	0.030	0.565
Fat	32.5	33.4	0.611	0.106
Protein	33.5	36.4	0.102	0.021
Lactose	33.4	35.0	0.398	0.246
Total solids	100	109	1.495	0.027
Composition, g/100g				
Fat	**4.19**	**4.35**	0.035	0.041
Protein	**4.51**	**4.67**	0.036	0.046
Lactose	4.49	4.50	0.099	0.955
Total solids	13.5	13.9	0.036	0.039
Milk free fatty acid, mevk/L	0.791	0.793	0.064	0.599
Feed efficiency, milk/DMI	0.540	0.565	0.032	0.060

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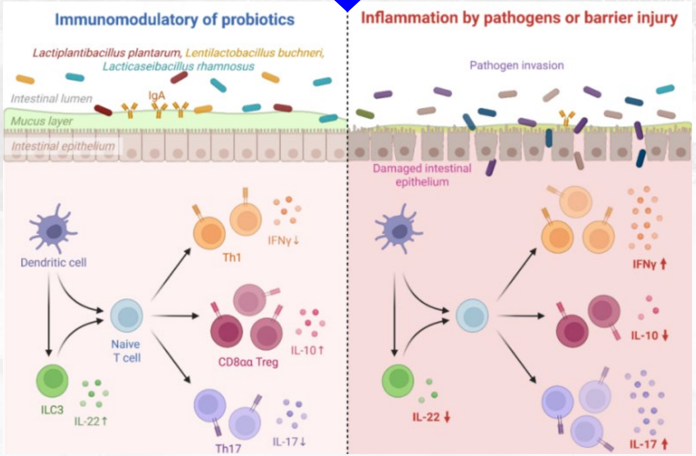
Probiotic-derived anti-inflammatory silage for animal health

- **Anti-inflammatory effects of probiotics mediate interactions with both intestinal epithelial cells and immune cells.**
- **Probiotics could also regulate dendritic cells to control the secretion of anti-inflammatory and proinflammatory cytokines, fostering the maintenance of immune homeostasis.**



Common clinical diseases in dairy cows□ mastitis, endometritis, ketosis, footrot *etc*.

- Cows intake silage every day
- Can we manipulate silage fermentation using certain probiotic strains to produce functional silage and prevent or alleviate inflammatory diseases of animals?
- Reduce using of antibiotics in animal production

Our pilot study results in dairy goats

- Feeding dairy goats with antioxidant-rich *L. plantarum* 24-7 inoculated alfalfa silage (Zhang et al. *J Anim Sci Biotechnol*., 2024)

Fig. 1 Immunoglobulins concentrations in the serum of dairy goats

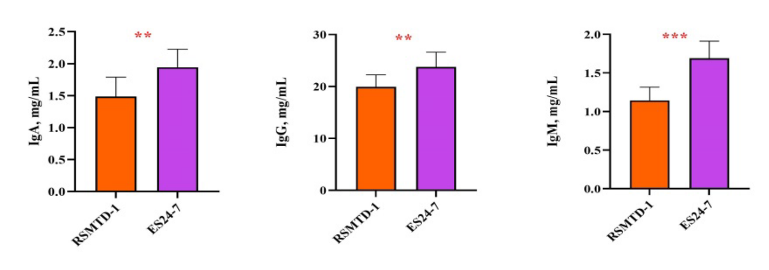
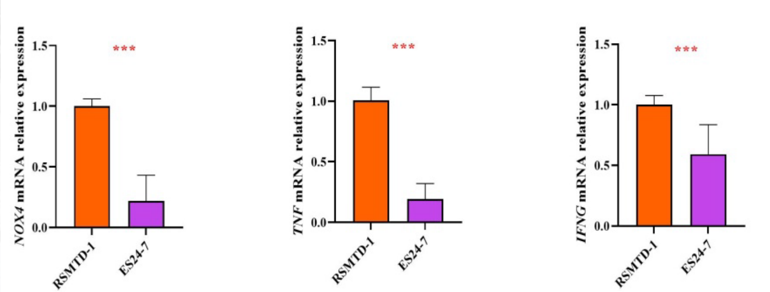
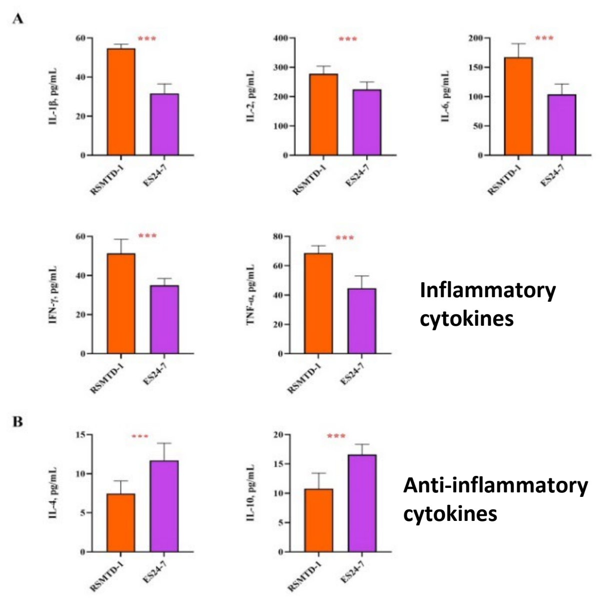


Fig. 3 Relative expression of inflammatory-related genes in the **mammary gland** sampled from dairy goats



Note *NOX4*, NADPH oxidase 4; *TNF*, tumor necrosis factor; *IFNG*, interferon gamma.

Fig. 2 Inflammatory and anti-inflammatory cytokines in the serum of dairy goats



Note IL-1*β*, interleukin 1*β*; IL-2, interleukin 2; IL-6, interleukin 6; IFN-*γ*, interferon-*γ*; TNF-*α*, tumor necrosis factor-*α*; IL-4, interleukin 4; IL-10, interleukin 10.

Feeding dairy goats with feruloyl esterase producing -*L. plantarum* A1 inoculated alfalfa silage

Ferulic acid in alfalfa silage can suppress inflammation（Li et al.□ *Anim Nutrition*., 2022）

Fig. 1. Serum immunoglobulin concentration from dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* A1

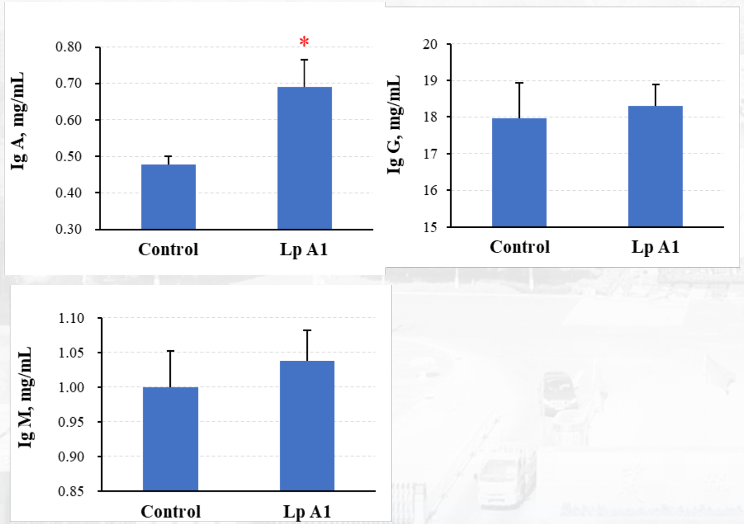
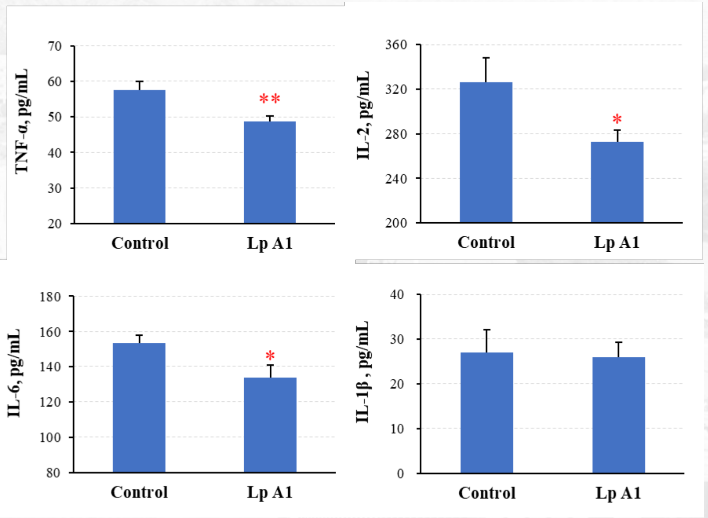


Fig. 2. Serum proinflammatory factors concentration from dairy goats fed diets including alfalfa silage inoculated with *L. plantarum* A1



Note: TNF-*α*, tumor necrosis factor-*α*; IL-2, interleukin 2; IL-6, interleukin 6□ IL-1β, interleukin 1β.

Feeding dairy goats with γ-aminobutyric acid-producing *L. buchneri* inoculated alfalfa silage

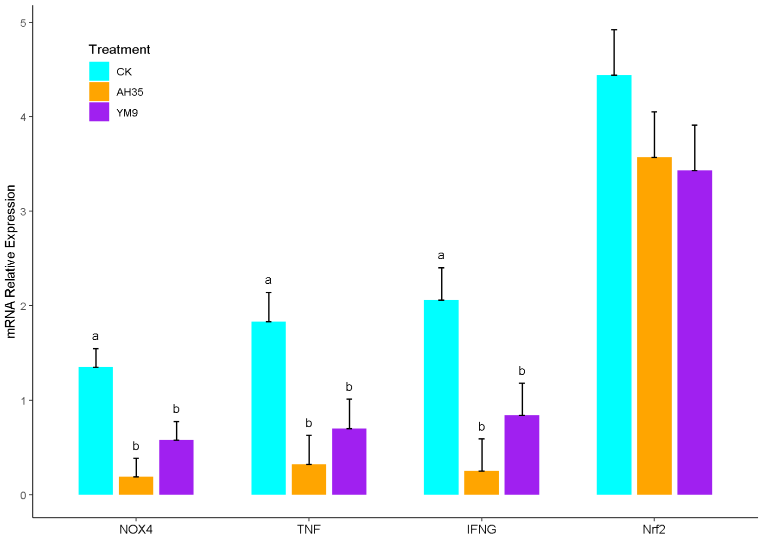
γ-aminobutyric acid(GABA)□ non-protein amino acid□ can alleviate animal stress, and promote feed intake.

Table 1. Milk yield and milk composition of dairy goats fed diets including alfalfa silage inoculated with *L. buchneri*

Item	Treatments			SEM	p-values
	Control	AH35	YM9		
DMI, kg/d	1.84^{a}	1.78^{b}	1.66^{c}	0.010	<0.001
Milk production					
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
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
TS, g/kg DM	39.7^{c}	43.8^{b}	**48.0^{a}**	1.130	<0.001
SNF, g/kg DM	31.5^{b}	33.2^{b}	**36.9^{a}**	0.879	<0.001
GABA, μmol/L	114^{b}	111^{b}	**152^{a}**	3.99	<0.001

AH35, YM9: γ-aminobutyric acid-producing *L. buchneri* strains

Fig. 1. Mammary gland proinflammatory/oxidant genes expression



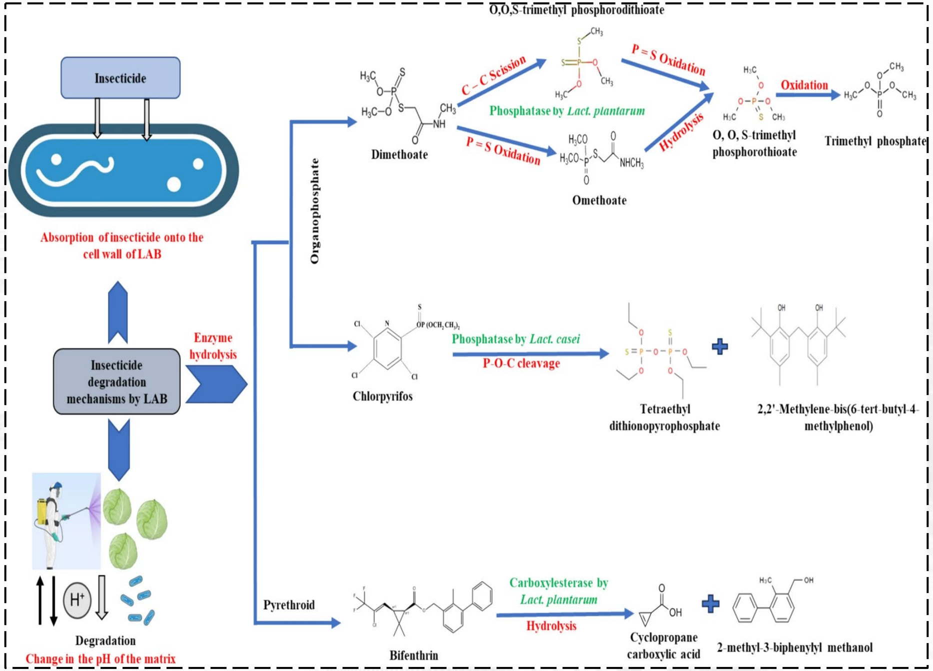
Note□ *NOX4*, NADPH oxidase 4; *TNF*, tumor necrosis factor; *IFNG*, interferon gamma.

Mitigating contaminants in silage by inoculating screened LAB

- **Bio-control of pesticides in silage**
- **Bio-control of antibiotic-resistant genes in silage**

Bio-control of pesticides in silage

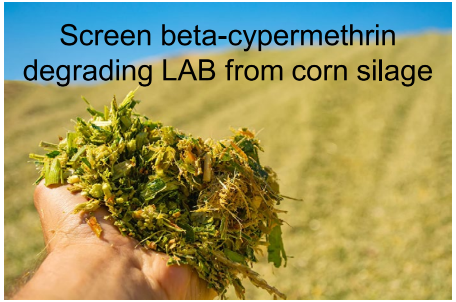
Different LAB inoculants for the degradation of pesticides in silage				
LAB strains	Pesticides	IRAC MoA classification	Source	References
L. plantarum 1.0315, *L. plantarum* 1.0624, *L. plantarum* 1.0622	Phorate and chlorpyrifos	Organophosphates 1B	Corn silage	(Zhang et al., 2016)
L. casei WYS3	Chlorpyrifos	Organophosphates 1B	Rice straw silage	(Wang et al., 2016)
***L. pentosus* 3–27**	**Beta-cypermethrin**	**Pyrethroids 3A**	**Alfalfa silage**	**(Liu et al., 2022)**



Mechanisms employed by LAB in insecticide residue degradation. (Kiruthika, K., et al. 2025. Probiotics & Antimicro. Prot. 17, 81–102. doi: 10.1007/s12602-024-10298-0.)

Bio-control of the pesticide beta-cypermethrin in silage

(Liu et al., *Journal of Hazardous Materials*, 2022)



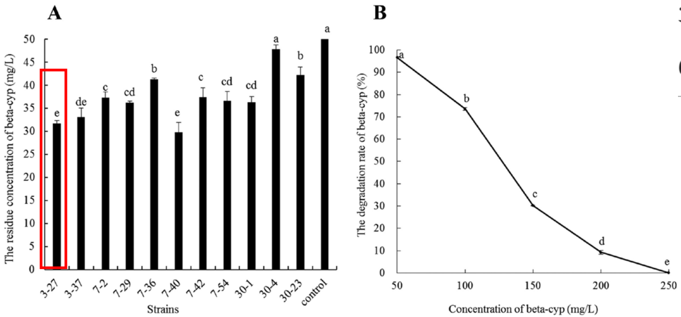


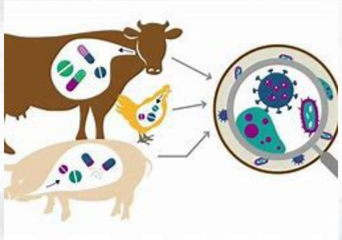
Table 1. Degradation rate (%) of beta-cyp in alfalfa silage with different dry matter contents during ensiling.

	Treatment[b] (35% DM)		Treatment[b] (43% DM)			*P* value[d]		
D[a]	C	LP	C	LP	SEM[c]	T	DM	T × DM
3	22.9*	26.2*	21.3*	21.6*	0.005	0.147	0.007	0.227
7	28.0*	33.0*	21.8*	26.9*	0.005	< 0.001	< 0.001	0.46
14	26.2*	33.3*	17.2*	28.9*	0.003	< 0.001	< 0.001	0.001
30	33.3*	41.3*	33.1*	37.4*	0.003	< 0.001	0.008	0.014
60	54.0*	57.2*	39.7*	52.2*	0.004	< 0.001	< 0.001	< 0.001

- A novel beta-cypermethrin-degrading ***L. pentosus* 3-27** was screened from silage.
- The Lp 3-27 **degraded 96% of beta-cyp** (50 mg/L) in MSM medium.
- A higher beta-cyp degradation was observed in silage with 35% DM versus 43% DM.

Bio-control of antibiotic-resistant genes (ARGs) in silage

- Antibiotic resistance is one of the greatest public health challenges worldwide.
- Annual mortality exceeding 10 million from antimicrobial resistance (Anon EU, 2010)
- Recently, ARGs have been continuously found in **antibiotic-resistant microbes** isolated from dairy products (Verónica et al., 2015).
- In addition, ARGs such as Tetracycline, Streptomycin, β-lactam resistant genes, were also found in beef (Yu et al., 2017).
- Silage as a fermented and major feed source for ruminants, it is essential to investigate the dissemination of ARGs in silage, and to mitigate the occurrence and spread of ARGs in silage.



Characteristics of ARGs in corn silage from various climate zones in China

Xu et al., *Environmental Pollution*, 2023

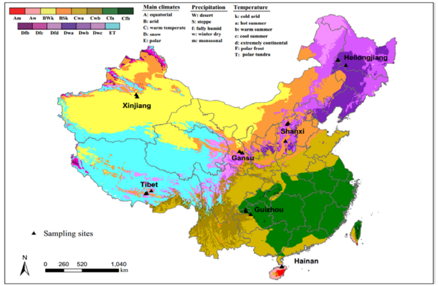


Fig. 1 The sampling sites of corn silages in typical climate zones of China

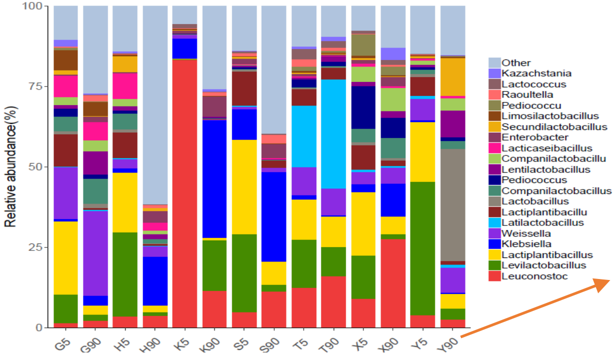


Fig. 2 Bacterial community composition of corn silage fermented for 5 and 90 days.

G, Guizhou province

X, Xinjiang province;

T, Tibet province;

S, Shanxi province

H, Heilongjiang province

K, Hainan province

Y, Gansu province.

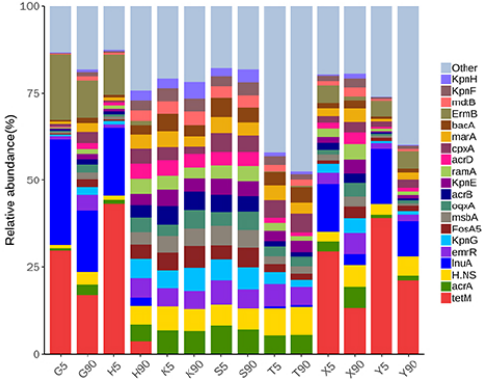


Fig. 3 ARGs composition of corn silage fermented for 5 and 90 days

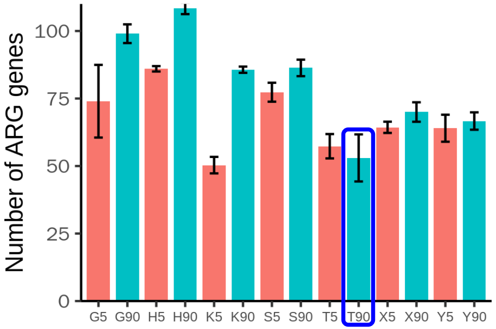


Fig. 4 ARGs numbers in corn silage fermented for 5 and 90 days

- Most of the bacteria responsible for the silage fermentation were the hosts to the ARGs
- There were 5 high-risk ARGs (*tetM*, *bacA*, *SHV-1*, *dfrA17*, and *QnrS1*) in silage from different climate zones, and the ***tetM*** (Tetracycline resistant gene) was the most prevalent high-risk ARG.

Effects of storage temperatures and inoculants on transmission of high-risk ARGs in corn silage

(Xu et al., *Science of Total Environment*, 2024)

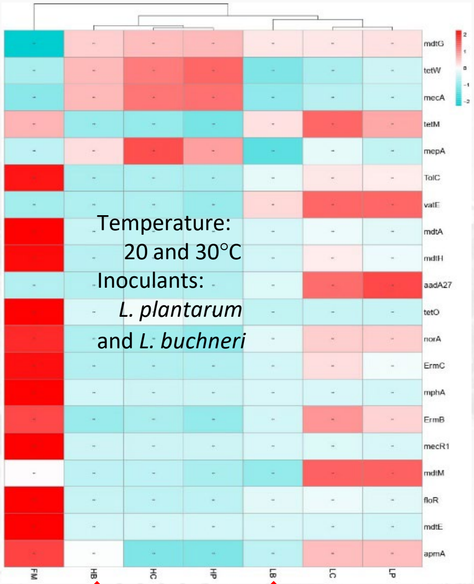


Fig.1 Heatmap of high-risk ARGs in corn silage

FM, fresh corn; LC, control silage at 20°C; LP, *L. plantarum* silage at 20°C LB, silage inoculated with *L. buchneri* silage at 20°C; HC, control at 30°C; HL, *L. plantarum* at 30°C; HB, *L. buchneri* at 30°C.

Results:

※*Lentilactobacillus buchneri* and high storage temperature reduce high-risk ARGs in corn silage.

※ The macrolide and tetracycline were the main ARGs in corn silage.

Effects of inoculants and dry matter on transmission of high-risk ARGs in corn silage

(Zhang et al., *Journal of Hazardous Materials*, 2024)

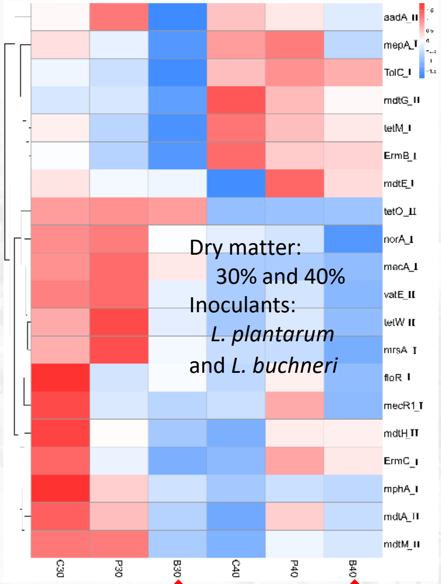


Fig.2 Heatmap of high-risk ARGs in corn silage

C30, control with 30% DM; P30, *L. plantarum* silage with 30% DM; B30, *L. buchneri* silage with 30% DM; C40, control with 40% DM; P40, *L. plantarum* silage with 40% DM, B40, *L. buchneri* silage with 40%DM.

Results:

※*Lentilactobacillus buchneri* and low dry matter reduce high-risk ARGs in corn silage.

※ The macrolide and tetracycline were the main ARGs in corn silage.

Effects of inoculants on transmission of ARGs in alfalfa silage

(Zhang et al., *Journal of Hazardous Materials*, 2023)

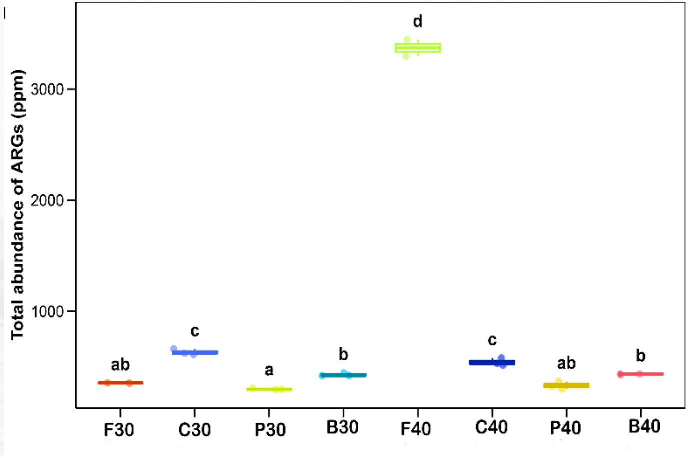


Fig. 1. ARGs abundances in LAB inoculated alfalfa silage
F, fresh alfalfa; C, control, P, *L. plantarum*; B, *L.buchneri*; 30, 30%DM;40, 40% DM.

- Both *L. plantarum* and *L. buchneri* reduced the ARGs abundance in alfalfa silage;
- *L. plantarum* had a better effect than *L. buchneri.*

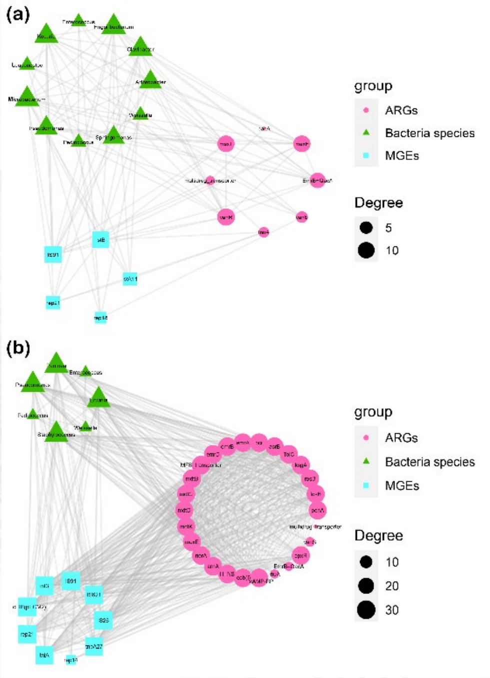


Fig. 2. Network analysis reveals the co-occurrence relationships among antibiotic resistance genes (ARGs), mobile genetic elements (MGEs) and microbes (at the genus level) in alfalfa silage **(a)** 30% DM and **(b)** 40% DM.

Conclusion

- **There are numerous opportunities in manipulation of silage fermentation for animal health**

1□ Developing LAB with effective decontamination ability to suppress pollutants in silage

2□ Developing specific probiotic LAB to prepare probiotic silage with antioxidant, anti-inflammatory, antimicrobial, immune stimulative and other health beneficial profiles.

- **There are great potentials to prevent and alleviate some clinical diseases of animals through feeding probiotic silages**

- **There are infinite possibilities in manipulation of silage fermentation through novel LAB when connecting silage to:**

1) Environmental sustainability
2) Animal performance, animal health and welfare, animal foods quality and safety, and even human being.

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THANK YOU FOR YOUR ATTETNTION

July 21, 2025