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

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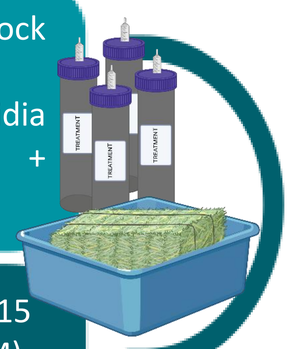
INTRODUCTION

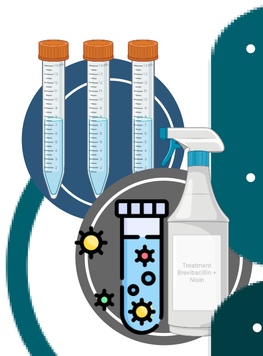
- Forage contamination by *Clostridia* cause unfavourable silage fermentation, and financial losses in the dairy industry (Driehuis et al., 2018).
- Heat-resistant *Clostridia* spores continue to exist in preserved silage, causing silage quality deterioration and subsequent problems in cheese production (Avila et al, 2020)
- Bacteriocins, as natural antimicrobial peptides, inhibiting or eliminating particular bacterial populations 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 Clostridia population in silage and their impact on silage quality and nutritive value.

METHODOLOGY

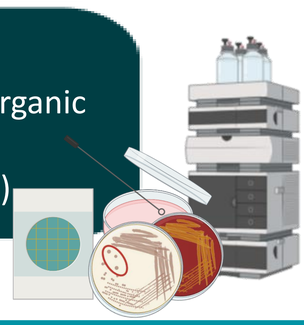
- Forage: third-cut alfalfa in a randomized block design (4 blocks).
- Treatments: Control (no additive), Clostridia (inoculated), Bacteriocin-treated, Clostridia + Bacteriocin-treated.



- Bacteriocins: Nisin (60 µg/g FM), Enterocin (15 µg/g FM), Brevibacillin (25 µg/g FM), Nisin+Brevibacillin (7 µg/g FM).
- Applied to non-inoculated silage or after inoculation with Clostridia inoculation.



- Silage Sampling: Days 0, 3, 100.
- Analysis: microbial count and diversity, organic acids concentrations, ammonia and pH.
- Statistical analysis: Dunnett's test ($P \leq 0.05$)



RESULTS & DISCUSSION

- Non-inoculated forage: bacteriocin treatments significantly reduced clostridia counts, with clostridia becoming undetectable in all treated silages by Day 100.
- Clostridia-inoculated forage: bacteriocin treatments significantly suppressed clostridia growth compared to the silage inoculated with clostridia.

Table 1. Effects of Bacteriocin treatments on clostridia counts (log CFU/g FM) in silage at Day 0, Day 3, and Day 100.

	Control	Nisin	Enterocin	Brevibacillin	Nisin+ Brevibacillin	SEM	Clostridia	Clostridia+ Nisin	Clostridia+ Enterocin	Clostridia+ Brevibacillin	Clostridia+ Nisin+ Brevibacillin	SEM
Day0	5.958	2.107*	1.765*	1.955*	2.384*	0.087	6.909	3.223*	2.526*	2.091*	2.756*	0.119
Day3	6.393	1.321*	0.000*	0.000*	1.043*	0.078	7.948	4.063*	2.033*	4.316*	2.035*	0.2025
Day100	5.331	0.000*	0.000*	0.000*	0.000*	0.053	6.804	4.328*	3.519*	5.263*	5.745*	0.086

* Different from control at $P \leq 0.05$

Population of Lactic acid bacteria (LAB):

- On Day 4: no significant differences across all treatments, indicating that Bacteriocins did not negatively impact the LAB population.
- On Day 100: growth significantly higher in bacteriocin-treated silages (except in Brevibacillin), showing stimulation of LAB growth.

Table 2. Effects of Bacteriocin treatments on lactic acid bacteria counts (log CFU/g FM) in silage at Day 0, Day 3, and Day 10.

	Control	Nisin	Enterocin	Brevibacillin	Nisin+ Brevibacillin	SEM	Clostridia	Clostridia+ Nisin	Clostridia+ Enterocin	Clostridia+ Brevibacillin	Clostridia+ Nisin+ Brevibacillin	SEM
Day 0	4.952	5.061	5.292	5.042	5.314	0.1161	4.850	4.656	5.453*	4.818	4.947	0.074
Day 3	10.138	9.933	9.473	9.814	9.696	0.2486	9.365	9.723	9.806	9.488	10.15*	0.206
Day 100	6.360	7.848*	7.127*	6.653	6.725*	0.0998	6.422	6.913	7.040	7.411	7.079	0.051

* Different from control at $P \leq 0.05$

Lactic acid concentrations in Day 100: greater in bacteriocin-treated silages; indicating enhanced fermentation efficiency and improved silage preservation.

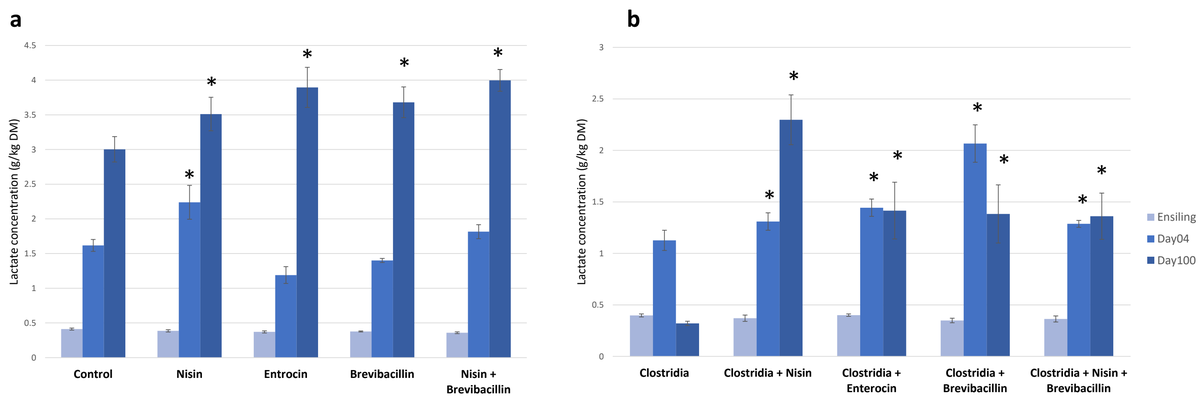


Figure 1. Effects of bacteriocin on lactic acid concentration in silage not inoculated and inoculated with clostridia; * significantly different from control at $P \leq 0.05$,

RESULTS & DISCUSSION

Ammonia concentration:

- Non-inoculated forage: no significant differences between treatments (Figure 2a).
- Clostridia-inoculated forage: bacteriocin treatments effectively reduced NH_3 concentration compared to the forage not treated with bacteriocins (Figure 2 b) .

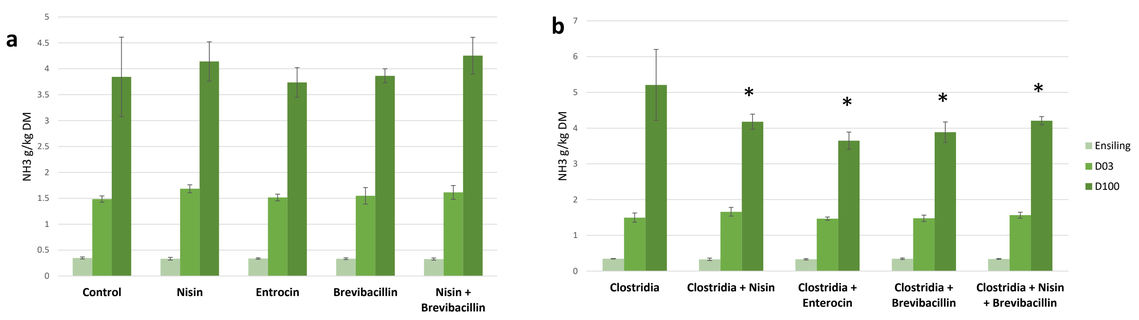


Figure 2. Effects of bacteriocin treatments on ammonia in silage not inoculated and inoculated with clostridia; * significantly different from control at $P \leq 0.05$,

Silage pH:

- Non-inoculated forage: no significant differences between treatments (Figure 2a).
- Clostridia-inoculated forage: Clostridia-inoculated silage maintained a higher final pH (Day 100), while all bacteriocin treatments maintained a lower pH.

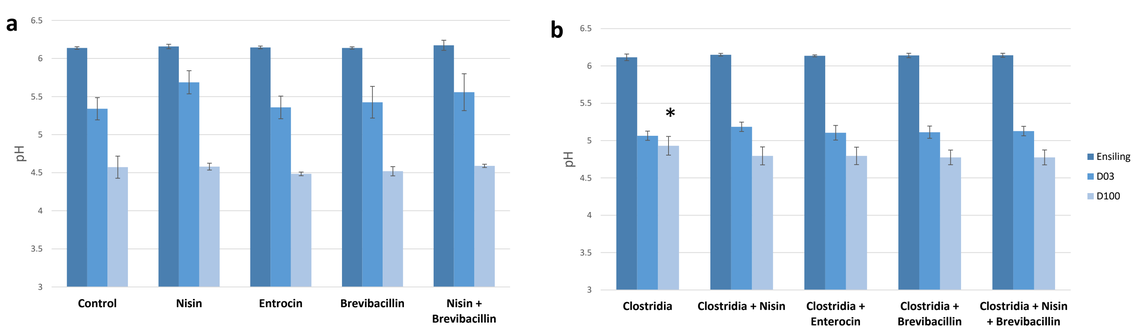


Figure 3. Effects of Bacteriocin Treatments on pH in Silage not inoculated and inoculated with clostridia

Bacterial Abundance:

- At Day 0, Bacilli and Bacteroidia were the dominant microbial families, with very low levels of Clostridia.
- By Day 100, Clostridia increased significantly ($P < 0.05$) in most treatments, indicating microbial spoilage.
- Silages treated with Bacteriocins effectively suppressed Clostridia growth.
- Bacilli and Bacteroidia remained consistently abundant throughout the fermentation period.

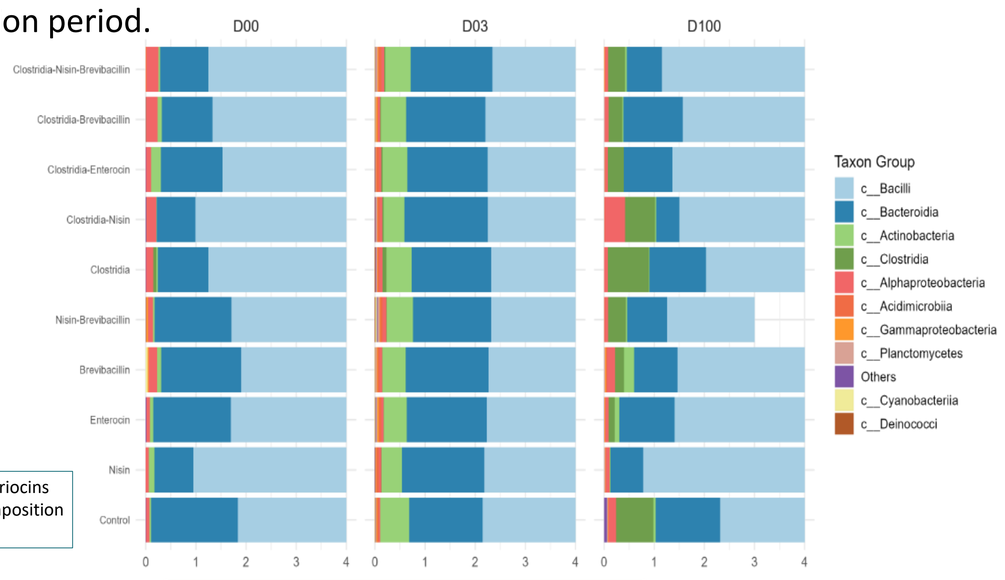


Figure 4. Effect of Bacteriocins on Bacterial Family Composition During Ensiling

CONCLUSION

In conclusion, This study demonstrates that Bacteriocins effectively suppress *Clostridia*, enhance fermentation quality, and support lactic acid bacteria populations in silage. Their ability to improve lactic acid production highlights their potential as natural preservatives. These results provide a foundation for the practical application of Bacteriocins in silage production.

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