Lactic acid bacteria inoculants as a tool for sustainable forage preservation and rumen fermentation efficiency

James A. Pickup[1], Icaro R. R. Castro[1], Angelika Borkowska[2], Fernanda G. Santos[1], Esperanza Fuertes[1], Nicholas J. Dimonaco[1], Philip Jones[2], **Mark Leggett[2]**, Sharon A. Huws[1]

[1]Queen's University Belfast, School of Biological Sciences, Institute for Global Food Security, Belfast, United Kingdom BT9 5DL.
[2]Volac International Ltd, Port Talbot, United Kingdom.

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).

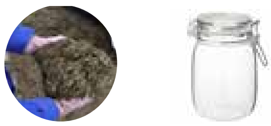
Objectives

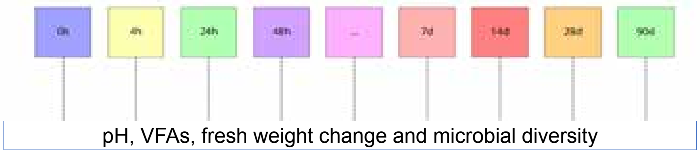
This study investigated the effects of three lactic acid bacteria (LAB) inoculants: (1) Ecosyl [*Lactiplantibacillus plantarum* MTD/1 (NCIMB 40027)]; (2) PJB1 [*Lentilactobacillus buchneri* PJB/1 (NCIMB 30139)], and (3) Ecocool (a blend of *Lactiplantibacillus plantarum with Lactiplantibacillus buchneri*) on the quality, microbial composition, and fermentation characteristics of perennial ryegrass (PRG)-based silages. Additionally, the impact of these inoculants on rumen fermentation was evaluated by measuring pH, gas production, methane emissions, volatile fatty acids (VFAs) and ammonia concentration.

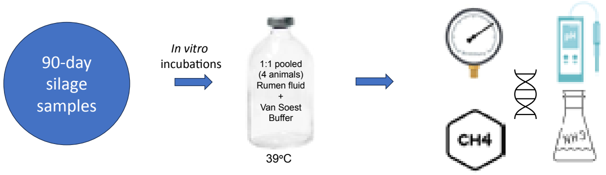
Material & Methods

The PRG was harvested, chopped 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 (~1×10^{6} CFU/g)
- Ecocool (~3×10^{5} CFU/g)
- PJB1 (~2×10^{5} CFU/g)







Active bacterial communities in the silage were analysed through full-length 16S rRNA gene sequencing using PacBio CCS technology, with the sequences processed using QIIME2. Any samples or species with ≤10 total counts and a relative abundance below 0.001% were excluded from the analysis. Taxonomic identities of amplicon sequence variants (ASVs) were assigned using the GreenGenes2 (2022.10) 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 was determined using PERMANOVA with 999 permutations (Vegan package, version 2.6-10) to assess the effects of treatment, time, and their interaction. Wilcoxon rank-sum tests and MaAsLin2 (version 1.8.0) were used to compare differences between groups. All statistical analyses were performed using the R programming language.

Results

In the silage, both Ecosyl and Ecocool maintained significantly lower pH than PJB1 and CON throughout the study (P<0.05; Fig. 1), had the highest lactic acid (LA) concentrations (P<0.01), and significantly higher proportion of LA in fermentation products relative to total fermentation acids (FA; Fig. 2). Significant weight changes were observed at 7, 28, and 90 days (P<0.05). Weight losses tended to be lower in Ecosyl treated silage, although losses were not different from the control after 90 days (Fig. 1). Weight losses were numerically higher in PJB1 and Ecocool silages after 90 days, possibly reflecting the heterofermentative fermentation of *L. buchneri*. Indeed, Ecocool and PJB1 silages contained significantly more acetic acid and 1,2-propanediol relative to control and Ecosyl silages, with a concurrent reduction in LA as a proportion of the total FA (Fig. 2), indicative of secondary LA fermentation by *L. buchneri* (Oude Elferink et al, 2001).

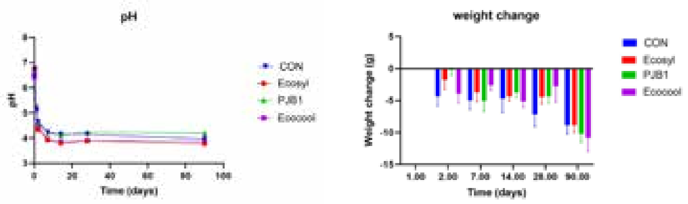


Figure 1: Change in pH and weight across the ensiling period by treatment: Control, Ecosyl, Ecocool and PJB1.

✓ mark.leggett@volac.com

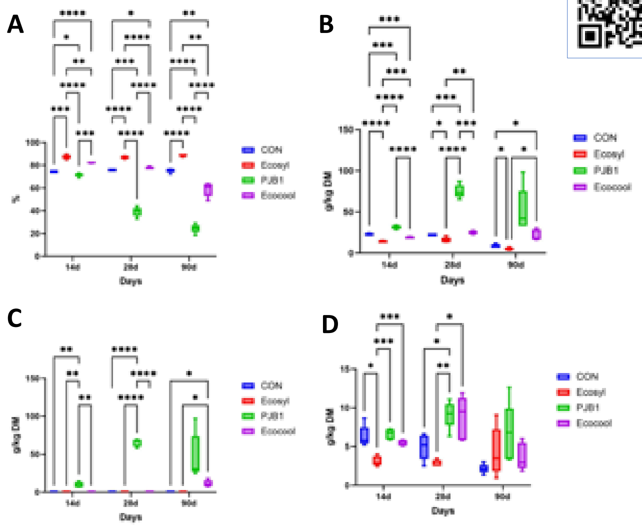


Figure 2: Major fermentation products at 14, 28 and 90 days ensiling. A) Lactic acid as % TFA, B) Acetic acid, C) 1,2-Propanediol, D) Ethanol

Microbial profiles of ensiled grass 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).

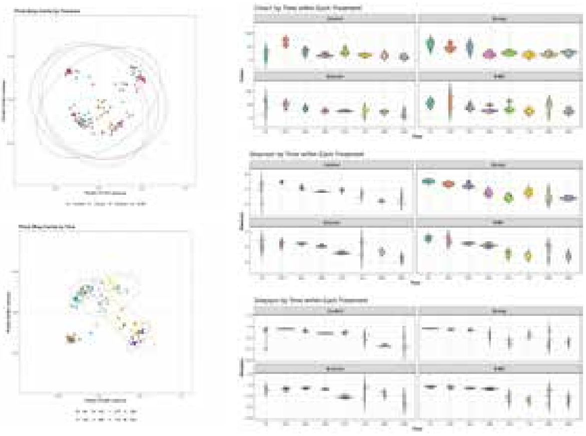


Figure 3: Alpha and Beta diversity indices. Treatment and time are indicated at each figure.

In the rumen, PJB1 reduced gas pressure (P<0.05). Inoculation with PJB1 also 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), highlighting its potential for mitigating greenhouse gas emissions from the rumen. Ecocool also tended to reduced methane levels relative to CON, but to a lesser extent; 4 h (33.82%; P<0.10), 24 h (23.39%; P<0.10), and 48 h (48.47%; P<0.10).

Table 1: Data from rumen *in vitro* fermentation of ensiled perennial ryegrass showing the data for 24- and 48-hour time points, means within a column not followed by the same letter are significantly different (p<0.05) from each other. 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	5.86^{a}	5.50^{a}	12.08^{a}	16.41^{a}	39.37^{a}	55.55^{a}	31.02^{a}	36.64^{a}	4260	3706	860	1409^{a}
Ecosyl	5.91^{a}	5.43^{a}	8.83^{b}	10.92^{b}	34.04^{a}	39.99^{ab}	30.76^{a}	36.70^{a}	3908	3310	878	1323^{a}
Ecocool	5.75^{a}	5.31^{ab}	7.68^{b}	8.16^{b}	30.15^{ab}	28.62^{ab}	29.56^{a}	34.76^{a}	4081	3393	991	1545^{ab}
PJB1	6.21^{b}	5.54^{ac}	16.31^{c}	18.07^{a}	13.80^{b}	26.87^{b}	20.62^{b}	29.82^{b}	4136	3607	1016	1142^{c}

Implications

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.

References

- Cheng, Q., Chen, L., Chen, Y., Li, P., & Chen, C. (2022). Effects of LAB inoculants on the fermentation quality, chemical composition, and bacterial community of oat silage on the Qinghai-Tibetan Plateau. Microorganisms, 10(4), 787. https://doi.org/10.3390/microorganisms10040787
- Kim, D., Lee, K. D., & Choi, K. C. (2021). Role of LAB in silage fermentation: Effect on nutritional quality and organic acid production—An overview. AIMS Agriculture and Food, 6(1), 216–234. https://doi.org/10.3934/agrfood.2021014
- Wang, Y.-L.; Wang, W.-K.; Wu, Q.-C.; Zhang, F.; Li, W.-J.; Yang, Z.-M.; Bo, Y.-K.; Yang, H.-J. The Effect of Different Lactic Acid Bacteria Inoculants on Silage Quality, Phenolic Acid Profiles, Bacterial Community and In Vitro Rumen Fermentation Characteristic of Whole Corn Silage. Fermentation 2022, 8, 285. https://doi.org/10.3390/ fermentation8060285
- Oude Elferink, S. J., Krooneman, J., Gottschal, J. C., Spoelstra, S. F., Faber, F., & Driehuis, F. (2001). Anaerobic conversion of lactic acid to acetic acid and 1, 2-propanediol by Lactobacillus buchneri. Applied and Environmental microbiology, 67(1), 125-132.