EFFECTS OF *LACTIPLANTIBACILLUS PLANTARUM* AND *PEDIOCOCCUS PENTOSACEUS* ON THE FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF GRASS/LEGUME SILAGE

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ABSTRACT

This study evaluated the effect of an inoculant (containing *Lactiplantibacillus plantarum* and *Pediococcus pentosaceus* in combination with xylanase enzyme) on the chemical composition, fermentation characteristics, and aerobic stability of grass/legume silage. The experiment was conducted using an in vitro mini-silo system in accordance with DLG guidelines, with silages analyzed after 90 days of fermentation. Compared with the untreated control, inoculated silage showed significantly higher contents of dry matter (DM), DM corrected for volatiles (DMc), crude protein (CP), and water-soluble carbohydrates (WSC), as well as reduced overall DM losses during storage. The inoculant significantly improved fermentation quality, as indicated by lower pH values, higher total acid concentrations, and increased levels of individual organic acids, particularly lactic acid. In addition, concentrations of undesirable fermentation products, including ammonia-N, alcohols, and butyric acid, were significantly reduced. Microbiological analysis showed higher lactic acid bacteria counts and reduced populations of yeasts, moulds, and clostridia. During the aerobic stability test, inoculated silage maintained lower pH, lower weight losses, and reduced spoilage microorganisms, resulting in significantly longer aerobic stability. Overall, inoculant application improved fermentation efficiency, preserved nutrients, suppressed undesirable microorganisms, and enhanced the aerobic stability of grass/legume silage.

Keywords: aerobic stability, grass/legume silage, bacterial strains, fermentation, dry matter losses

INTRODUCTION

Grass/legume mixtures are increasingly used in sustainable livestock production systems because they combine the high energy yield of grasses with the protein-rich biomass of legumes. Nevertheless, these mixtures may present challenges during ensiling due to differences in fermentability and buffering capacity (Muck *et al.*, 2021; Gao *et al.*, 2024). The application of additives is an effective strategy to reduce the risk of undesirable fermentation, thereby limiting dry matter (DM) losses and aerobic spoilage in silage. Therefore, the use of live lactic acid bacteria (LAB) may play an important role in optimizing fermentation processes and improving the aerobic stability of mixed forages. Xylanase enzymes can be included in some silage inoculants to hydrolyze hemicellulosic components of the plant cell wall, thereby increasing the release of fermentable sugars available for LAB. The combination of LAB and enzymes is known for rapid lactic acid production and the ability to accelerate pH decline during the early stages of fermentation (Ávila and Carvalho, 2020; Kung *et al.*, 2022). In addition to fermentation quality, the aerobic stability of silage during feed-out is an important factor influencing feed hygiene and nutrient retention (Wilkinson and Davies, 2019). The objective of this study was to evaluate the effects of *Lactiplantibacillus plantarum* and *Pediococcus pentosaceus* in combination with a xylanase enzyme on the fermentation characteristics and aerobic stability of perennial ryegrass/red clover/timothy silage.

MATERIAL AND METHODS

The tested additive was Josilac Classic (Josera GmbH, Germany), contained *Lactobacillus plantarum* strains (NCIMB 30083, NCIMB 30084) and *Pediococcus pentosaceus strains* (DSM 23689, DSM 23688) with a concentration of 1×10^{11} CFU g^{-1}, xylanase enzyme (43,000 HET g^{-1}), and dextrose as a carrier. The application rate was 6×10^5 CFU g^{-1} forage. The product was stored below 5 °C until use. A first-cut mixture of perennial ryegrass, red clover and timothy was harvested, wilted for 8–12 h to >30% dry matter (DM), and chopped to 2–3 cm length. Glass mini-silos (3 L) with gas-venting caps were used as experimental units. The inoculant was diluted in unchlorinated water to 6×10^8 CFU ml^{-1} and applied at 4 ml kg^{-1} forage to obtain a final dose of 6×10^5 CFU g^{-1} forage (JC). Control (C) forage received 4 ml kg^{-1} sterile unchlorinated water. Each 3-L silo was filled with 1.68 kg forage (36.6% DM) to reach a density of 1 kg DM per 5 L volume. Silos were stored at 20–21 °C for 90 days. After 90 days, silos were opened and analyzed for fresh matter and DM losses, pH, lactic acid, volatile fatty acids (acetic, propionic, butyric), ammonia-N, and alcohols. Counts of lactic acid bacteria, yeasts, and molds were determined at ensiling and at silo opening. After 90 days of storage, silages were exposed to air for 14 days. Aerobic stability was defined as the time required for silage temperature to increase by more than 3 °C above ambient temperature. Data were analyzed using one-way ANOVA in a randomized complete block design (5 replications per treatment) with SAS Proc GLM (version 8.02).

RESULTS AND DISCUSSION

Table 1 shows that the inoculant treatment (JC) significantly affected the fermentation quality, microbial characteristics, and aerobic stability of grass/legume silage compared with the control (C). The DM and volatile-corrected DM content was by 2.6% and by 2.1%, respectively, higher ($p < 0.01$) in JC than in C silage. Crude protein was by 3.9% higher ($p < 0.01$) in JC silage, whereas crude fibre concentration decreased by 15.1% ($p < 0.01$) compared with C ($p < 0.01$). Xylanase can promote a faster decline in pH, enhance fiber degradation, and potentially improve nutrient preservation and aerobic stability during ensiling (Muck *et al.*, 2021). The WSC concentration retained by 76.3% higher ($p < 0.01$) in the inoculated silage compare with non-inoculated C silage. Total fermentation acids were 17.9% higher in JC ($p < 0.01$), mainly due to a 25.4% increase in lactic acid ($p < 0.01$). Butyric acid decreased by 2.6-fold in JC ($p < 0.01$), whereas acetic and propionic acids were not significantly affected by the inoculant treatment.

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Tab. 1: Effect of inoculant treatment on the fermentation and microbial characteristics, and aerobic stability of ensiled grass/legume

Variables	C	JC	LSD0.05	EMS	P
DM, g/kg	336.3b	345.2a	5.170	12.567	0.004
DM*, g/kg	348.3b	355.7a	5.118	12.314	0.010
Crude protein, g/kg DM	151.1b	157.0a	4.633	10.093	0.019
Crude fibre, g/kg DM	207.6a	176.2b	13.881	90.581	0.001
WSC, g/kg DM	17.7b	31.2a	9.771	44.885	0.013
Total acids, g/kg DM	72.45b	85.42a	7.046	23.337	0.003
Lactic acid, g/kg DM	56.52b	70.89a	6.327	18.821	0.001
Acetic acid, g/kg DM	14.99a	13.89a	2.248	2.376	0.292
Butyric acid, g/kg DM	0.47a	0.18b	0.131	0.008	0.001
Alcohols, g/kg DM	11.36a	7.04b	1.356	0.865	<0.0001
N-NH3, g/kg total N	46.28a	32.51b	5.710	15.326	0.0005
pH after 3 d.	4.82a	4.65b	0.068	0.002	0.0004
pH after 90 d.	4.40a	4.27b	0.059	0.002	0.0007
DM loss, g/kg	64.95a	42.33b	8.936	37.543	0.0004
Yeast, log10 CFU/g	1.99a	1.06b	0.157	0.011	<0.0001
Moulds, log10 CFU/g	1.74a	1.00b	0.115	0.006	<0.0001
Aerobic stability test					
pH at the end of AST (14 d.)	8.64a	4.94b	0.554	0.144	<0.0001
Fresh weight loss during AST, %	4.18a	2.74b	0.619	0.180	0.0007
Yeast after AST, log10 CFU/g	7.34a	4.85b	0.542	0.138	<0.0001
Moulds after AST, log10 CFU/g	9.00a	5.45b	0.762	0.273	<0.0001
Aerobic stability, hours	186b	256b	43.311	1743.00	0.001

*Dry matter corrected for volatiles; [a, b]Means with different superscript letters in a line indicate significant differences of $p < 0.01$

Alcohols and ammonia- N concentrations were by 38.0% and by 23.9%, respectively lower ($p < 0.01$) in JC silages compared with C silage. The inoculant treatment reduced silages pH values, decreasing by 0.17 units and by 0.13 units ($p < 0.01$) after 3 and after 90 days fermentation, respectively. Inoculant treatment reduced DM losses by 34.8% ($p < 0.01$). Regarding microbial counts, yeasts and moulds were reduced by 46.7% and 42.5%, respectively ($p < 0.01$). During the aerobic stability test, JC silage showed markedly mitigated aerobic deterioration: pH at the end of AST was 42.8% lower ($p < 0.01$), fresh weight losses decreased by 34.4% ($p < 0.01$), yeasts and moulds after AST were reduced by 33.9% and 39.4%, respectively ($p < 0.01$). Aerobic stability increased by 37.6% (256 vs. 186 h; $p < 0.01$).

CONCLUSION

The combined inoculant treatment significantly improved silage fermentation quality and microbial profile. It increased DM, crude protein, WSC, lactic acid, and LAB counts, while reducing crude fibre, butyric acid, alcohols, ammonia-N, DM losses, yeasts, moulds, and clostridia. In addition, the inoculated silage showed markedly reduced silage aerobic deterioration, with lower pH, reduced spoilage microorganisms, and longer stability time.

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