INFLUENCE OF *LACTOBACILLUS PLANTARUM* AND DANDELION ON FERMENTATION QUALITY, BIOACTIVE RETENTION AND MICROBIAL COMMUNITY OF MULBERRY LEAF SILAGE IN CHINA

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INTRODUCTION

Mulberry leaves are recognized for their high crude protein content (18–25% DM) and excellent digestibility, making them a promising and sustainable feed resource for ruminants. However, their high moisture content and strong buffering capacity often impair proper silage fermentation. This study aimed to address these limitations by applying Lactobacillus plantarum (LP) inoculation and dandelion (D) supplementation, either individually or in combination (LP-D). It was hypothesized that these additives would enhance fermentation efficiency by lowering pH and increasing lactic acid production, while also promoting the preservation and enrichment of bioactive compounds, reducing anti-nutritional constituents, and stabilizing the microbial community during ensiling and subsequent aerobic exposure. The objective was to evaluate the effects of LP and D on the fermentation characteristics, bioactive compound retention, and microbial dynamics of mulberry-leaf silage after 30 and 45 days of ensiling followed by 4 days of aerobic exposure.

MATERIAL AND METHODS

Fresh mulberry leaves containing approximately 50% moisture were divided into four treatment groups: control (CK), Lactobacillus plantarum (LP; 5% inoculum, 10^8 CFU·mL^{-1}), dandelion (D; 5% hydrated dandelion), and a combined treatment of LP and D (LP-D), with three replicate bags per treatment. The samples were ensiled for 30 and 45 days and subsequently subjected to 4 days of aerobic exposure. Parameters evaluated included pH, ammonia nitrogen (mg·kg^{-1} DM), dry matter (DM) loss (%), water-soluble carbohydrates (WSC), crude protein (CP), and organic acids determined through HPLC. Bioactive components such as tartaric acid, flavonoids, polyphenols, polysaccharides, alkaloids, and antioxidant activity (DPPH) were also quantified. Structural and anti-nutritional constituents including cellulose, hemicellulose, lignin, phytates, and tannins were measured. Microbial community composition was assessed through 16S rRNA gene sequencing and quantitative PCR analysis. Statistical evaluation was performed using one-way ANOVA followed by Fisher's least significant difference test in SPSS, with significance accepted at $P < 0.05$.

RESULTS

Both LP and LP-D treatments significantly improved fermentation quality compared with CK ($P < 0.001$). The LP-D combination produced the lowest pH (4.21 vs. 4.68 in CK; $P < 0.001$), lowest ammonia-N concentration (47.3 vs. 72.4 mg·kg^{-1} DM; $P < 0.001$), and lowest dry matter loss (8.6% vs. 12.5%; $P < 0.001$). Lactic acid concentration was highest in LP-D silage (5.82 g·kg^{-1} FM), exceeding LP and D treatments ($P < 0.01$), while butyric acid remained undetectable across treatments ($P > 0.05$). Dandelion supplementation significantly increased flavonoids (3.85 mg·g^{-1}), polyphenols (6.12 mg·g^{-1}), and antioxidant capacity (72.3%) compared with CK ($P < 0.01$). Anti-nutritional constituents including phytates and tannins decreased significantly in LP-D silage after 30 days ($P < 0.05$). During aerobic exposure, Enterobacter counts increased in CK but remained significantly lower in LP and LP-D treatments ($P < 0.01$). Microbial sequencing showed that LP and LP-D reduced α-diversity indices (Shannon index; $P < 0.05$) and increased Lactobacillus abundance ($P < 0.001$). Lactobacillus abundance was negatively correlated with pH and anti-nutritional factors ($P < 0.01$) and positively correlated with lactic acid concentration ($P < 0.01$).

CONCLUSION

It was concluded that the use of LP, especially in combination with dandelion, effectively improved the fermentation quality of mulberry-leaf silage by promoting LAB dominance, elevating lactic acid concentration, minimizing proteolysis and dry matter loss, preserving and enhancing antioxidant bioactive compounds, and lowering anti-nutritional components, thereby improving overall digestibility and nutritive value. Dandelion functioned as a beneficial phytogenic additive that contributed to improved aerobic stability. The most suitable practice was LP-D treatment with approximately 30 days of ensiling for optimal silage quality and bioactive preservation. These results encourage the use of combined microbial and plant-based additives to produce high-quality preserved feed, while further research is recommended to evaluate animal performance, in-vitro rumen fermentation responses, and economic feasibility under farm conditions.

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