Effects of Different Additives on the Fermentation Quality of Hybrid Paper Mulberry Silage (Postprint)

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Abstract

This study investigated the effects of molasses, enzyme-bacterial complex preparation, and preservatives on the fermentation quality of hybrid paper mulberry silage, providing technical reference for the development and utilization of novel woody feed resources. The experiment comprised five treatments: control (no additive), mold complex preparation (group M), molasses (group TM), mold complex preparation + molasses (group MTM), and preservative (group FFJ), each with three replicates. The fermentation duration was 60 days. The results demonstrated: 1) Compared with the control, all additive groups exhibited significantly reduced pH ($P<0.05$) and significantly increased lactic acid content ($P<0.05$), thereby improving fermentation quality to varying extents. 2) Groups M, MTM, and FFJ significantly reduced the ammonia nitrogen/total nitrogen ratio relative to the control; notably, group FFJ displayed significantly higher dry matter content ($P<0.05$) while maintaining significantly lower pH and ammonia nitrogen/total nitrogen ratio ($P<0.05$) than all other groups. 3) Groups M and MTM significantly decreased acid detergent fiber content compared with the control ($P<0.05$). In summary, supplementation of hybrid paper mulberry silage with molasses, enzyme-bacterial complex preparation, and preservatives markedly enhanced both fermentation quality and nutritional value. Groups MTM and FFJ maintained lower pH levels and better preserved crude protein and dry matter, with group FFJ demonstrating the optimal effect, whereas group MTM offered a more economical alternative.

Full Text

Influence of Different Additives on Fermentation Quality of Hybrid Paper Mulberry Silage

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Abstract

This study investigated the effects of molasses, enzyme-bacteria composite preparation, and preservatives on the fermentation quality of hybrid paper mulberry silage to provide technical reference for the development and utilization of new woody feed resources. Five treatment groups were established: control (no additive), enzyme-bacteria composite preparation (M group), molasses (TM group), enzyme-bacteria composite preparation + molasses (MTM group), and preservatives (FFJ group), with three replicates per group. Fermentation lasted for 60 days. The results showed that: (1) Compared with the control group, all additive treatments significantly reduced pH ($P < 0.05$) and significantly increased lactic acid content ($P < 0.05$), thereby improving fermentation quality to varying degrees. (2) The M, MTM, and FFJ groups significantly reduced ammonia nitrogen/total nitrogen ratio compared with the control ($P < 0.05$). The FFJ group exhibited significantly higher dry matter content ($P < 0.05$) and significantly lower pH and ammonia nitrogen/total nitrogen ratio ($P < 0.05$) than all other groups. (3) The M and MTM groups significantly reduced acid detergent fiber content compared with the control ($P < 0.05$). In conclusion, adding molasses, enzyme-bacteria composite preparation, and preservatives to hybrid paper mulberry silage significantly improved both fermentation quality and nutritional value. The MTM and FFJ groups maintained lower pH and better preserved crude protein and dry matter, with the FFJ group showing the best overall results, though the MTM group offered a more cost-effective alternative.

Keywords: hybrid paper mulberry; silage; additives

Introduction

Feed resource shortage represents a significant constraint on the development of animal husbandry. Developing green, high-quality, and economical woody feed resources is crucial for China' s livestock industry. Paper mulberry (*Broussonetia papyrifera* (L.) Vent.), a dioecious deciduous tree belonging to the Moraceae family, is known regionally as "chushu," "maye tree," or "cutao tree." Hybrid varieties exhibit strong adaptability, tolerance to drought and cold, heat and humidity, extensive root systems, high biomass, and excellent regrowth capacity after cutting. Paper mulberry leaves contain over 20% crude protein [1], comparable to alfalfa, and are rich in amino acids, vitamins, and trace elements, making them a highly valuable unconventional forestry protein feed resource. Additionally, paper mulberry serves as a traditional Chinese medicinal herb, containing various flavonoids, alkaloids, polysaccharides, and unsaturated fatty acids with demonstrated pharmacological activities including antimicrobial, antiviral, antitumor, hypoglycemic, and hypotensive effects, as well as immune enhancement and anti-aging properties [2]. The State Council Poverty Alleviation Office designated paper mulberry cultivation as one of ten targeted poverty alleviation projects in 2015, and China has intensified large-scale promotion efforts, with hybrid paper mulberry planting area expected to exceed 5 million hectares during the 13th Five-Year Plan period. Traditionally, rural farmers have fed paper mulberry leaves to pigs, cattle, and sheep. However, due to the complex molecular structure of paper mulberry proteins, untreated leaves exhibit low digestibility and utilization rates in livestock [3]. Research on the effects of different additives on fermentation quality of whole-plant hybrid paper mulberry silage remains limited [4]. This study examines the influence of various additives on fermentation quality to provide theoretical reference for large-scale cultivation and comprehensive utilization of hybrid paper mulberry.

Materials and Methods

1.1 Materials Silage material: Hybrid paper mulberry was harvested in July 2017 from a plantation in Daxing District, Beijing. Plants approximately 1.6 m in height were harvested whole using a forage harvester at a stubble height of 15-20 cm, chopped to 1-2 cm lengths (pH 7.35). Nutritional and microbial compositions are presented in Table 1 .

Molasses: A byproduct of sugar production, primarily sucrose, appearing as a reddish-brown viscous liquid with 50.01% dry matter content and 65.2% water-soluble carbohydrate content (dry matter basis), purchased from Xuzhou Yibang Environmental Protection Technology Co., Ltd.

Enzyme-bacteria composite preparation: Containing lactic acid bacteria, cellulase, xylanase, and -glucanase in powder form, purchased from Ningxia Xiasheng Industrial Group Co., Ltd.

Preservatives: A liquid preparation containing sodium propionate, sodium nitrite, and hexamethylenetetramine, purchased from German Aidekang Group.

1.2 Experimental Design and Silage Preparation The experiment comprised five treatment groups (Table 2). Required additives were dissolved in 200 mL distilled water, thoroughly mixed, and evenly sprayed onto 50 kg of fresh material. The control group received an equal volume of distilled water. Samples of 1,000 g were packed into polyethylene bags (24 cm × 40 cm), with three replicates per group, vacuum-sealed (Model DZ-280/2SD), and stored at room temperature for 60 days before opening. Fresh hybrid paper mulberry samples were collected during silage preparation and stored at -20 °C for subsequent analysis.

1.3 Analytical Methods

1.3.1 Nutritional Composition Analysis Silage samples were dried at 60 °C for 48 h and ground through a 1 mm sieve. Dry matter, crude protein, ether extract, and ash contents were determined using AOAC methods. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were analyzed using the Van Soest method [5].

1.3.2 Fermentation Quality Analysis After opening silage bags, 20 g of hybrid paper mulberry silage was mixed with 180 mL distilled water and homogenized for 1 min. The homogenate was filtered through four layers of gauze, and pH was measured using a Leici PHS-3C precision pH meter [6]. Ammonia nitrogen content was determined by the phenol-hypochlorite colorimetric method [7]. For silages containing sodium nitrite and hexamethylenetetramine, ammonia nitrogen values were corrected according to the maximum potential ammonia nitrogen production from these additives [8], as uncorrected values would overestimate protein hydrolysis when expressed as ammonia nitrogen/total nitrogen ratio [9].

Water-soluble carbohydrate content was measured using the anthrone-sulfuric acid method [10]. Buffering capacity was determined by hydrochloric acid and sodium hydroxide titration [11]. Lactic acid, acetic acid, propionic acid, and butyric acid contents were analyzed using a SHIMADZE-10A high-performance liquid chromatograph (KC-811 column; column temperature 50 °C; flow rate 1 mL/min; detection wavelength 210 nm; injection volume 5 L) [12].

1.3.3 Microbial Analysis Five grams of silage sample was mixed with 45 mL sterile water and serially diluted (10^{1} to 10). Plate spreading method was used for colony cultivation. Lactic acid bacteria were cultured on MRS medium at 37 °C under anaerobic conditions for 48 h; yeasts and molds on PDA medium at 30 °C under anaerobic conditions for 24 h; aerobic bacteria on NA medium at 30 °C under aerobic conditions for 24 h. Visible colonies were counted [13].

1.4 Data Processing and Analysis Experimental data were analyzed using SAS 9.2 statistical software. ANOVA was performed, and Duncan' s multiple range test was used for mean comparisons, with $P < 0.05$ as the significance threshold.

Results

2.1 Nutritional and Microbial Composition of Hybrid Paper Mulberry Before Ensiling The nutritional composition and epiphytic microbial populations of hybrid paper mulberry prior to ensiling are shown in Table 1. The material exhibited low dry matter (21.18% FW), water-soluble carbohydrate (4.18% DM), and ether extract (3.05% DM) contents, but high crude protein (18.55% DM) and buffering capacity (502.13 mE/kg DM). Lactic acid bacteria counts were 4.51 log CFU/g FW, below the recommended 5 log CFU/g FW threshold, while aerobic bacteria, yeasts, and molds were relatively abundant at 6.81 and 5.20 log CFU/g FW, respectively.

2.2 Nutritional Composition of Hybrid Paper Mulberry Silage As shown in Table 3 , the control group exhibited significantly lower crude protein content than all additive groups ($P < 0.05$). The FFJ group showed significantly higher dry matter content than other groups ($P < 0.05$), while the FFJ and MTM groups had significantly higher crude protein and ether extract contents ($P < 0.05$). The M group demonstrated significantly lower NDF and ADF contents compared with other groups ($P < 0.05$). No significant differences were observed in ash content among groups ($P > 0.05$).

2.3 Fermentation Quality of Hybrid Paper Mulberry Silage As presented in Table 4 , the control group had significantly higher pH than all additive groups ($P < 0.05$), with the FFJ group showing the lowest pH. All additive treatments significantly increased lactic acid content compared with the control ($P < 0.05$). The M group exhibited significantly higher acetic acid content than other groups ($P < 0.05$). No butyric acid was detected in any treatment. The FFJ group showed significantly lower ammonia nitrogen/total nitrogen ratio than all other groups ($P < 0.05$).

Discussion

3.1 Effects of Additives on Fermentation Quality of Hybrid Paper Mulberry Silage The hybrid paper mulberry harvested in this experiment was approximately 1.6 m tall with tender branches and leaves, resulting in low dry matter content. Successful silage fermentation typically requires abundant lactic acid bacteria and water-soluble carbohydrates, with lactic acid bacteria populations ideally exceeding 5 log CFU/g FW to promote adequate fermentation [14]. The hybrid paper mulberry material contained only 4.51 log CFU/g FW lactic acid bacteria and 4.18% water-soluble carbohydrates, combined with low dry matter, high crude protein, and high buffering capacity, classifying it as a difficult-to-ensile material.

In this study, the M, TM, and MTM groups significantly reduced pH and increased lactic acid content compared with the control, improving fermentation quality. All treatments exhibited lactic acid/total acid ratios above 70%, indicating lactic acid fermentation dominance. Chen et al. [15] reported that lactic acid bacteria addition significantly improved fermentation quality of whole-plant corn total mixed ration silage, with supplementary fermentation substrates (glucose, molasses) providing further enhancement. Similarly, Li et al. [16] found that glucose and molasses addition significantly reduced pH and increased lactic acid content in king grass silage, consistent with Lima et al. [17]. These effects primarily result from molasses and enzyme-bacteria composite preparations providing additional fermentation substrates or increasing lactic acid bacteria populations, thereby generating more lactic acid.

The control group' s high buffering capacity and low lactic acid production resulted in slow pH decline, failing to effectively inhibit proteolytic microorganisms. Combined with high moisture and protein content, this led to substantial protein degradation and elevated ammonia nitrogen production. Ammonia nitrogen in silage originates primarily from plant enzyme-mediated protein degradation and microbial utilization of proteins and amino acids [18-19], with the ammonia nitrogen/total nitrogen ratio reflecting the extent of protein degradation. The M, TM, and MTM groups maintained lower ammonia nitrogen/total nitrogen ratios, likely due to lactic acid bacteria inoculation or increased fermentation substrates promoting rapid pH decline, which inhibited plant proteases and microbial protein degradation [20].

The FFJ group received a mixed additive containing sodium propionate, sodium nitrite, and hexamethylenetetramine. Under acidic conditions, sodium propionate releases free propionic acid that strongly inhibits various molds, aerobic bacilli, and Gram-negative bacteria. Hexamethylenetetramine hydrolyzes to hippuric acid and formaldehyde, with formaldehyde causing pathogen protein denaturation and exerting non-specific antimicrobial effects. Consequently, the FFJ group exhibited the lowest pH, highest propionic acid/total acid ratio, minimal protein degradation, and greatest dry matter preservation. Chen et al. [9] reported similar results in smooth vetch silage treated with lactic acid bacteria and a mixture of sodium benzoate, sodium propionate, sodium nitrite, and hexamethylenetetramine, which reduced pH and ammonia nitrogen while increasing dry matter content, consistent with Lingvall et al. [21].

The absence of butyric acid in all treatments may be attributed to flavonoid compounds naturally present in paper mulberry. Reportedly, paper mulberry leaf extracts contain 273 mg/kg flavonoids [22], which exhibit strong antimicrobial activity. At 1.0% concentration, paper mulberry leaf extract demonstrates superior antimicrobial and preservative effects compared to 0.1% sodium benzoate, with concentrations of 0.5% and above inhibiting bacterial and fungal growth, indicating that paper mulberry flavonoids serve as effective natural preservatives [23].

3.2 Effects of Additives on Nutrient Content of Hybrid Paper Mulberry Silage Dry matter loss in silage begins with plant cell respiration, where aerobic microorganisms utilize carbon sources to produce water, heat, and carbon dioxide, thereby destroying nutrients. The addition of sodium propionate, sodium nitrite, and hexamethylenetetramine in the FFJ group accelerated pH decline, inhibiting plant protease and microbial degradation of proteins and amino acids, reducing dry matter loss, and increasing protein content. Similarly, enzyme-bacteria composite preparation and molasses addition significantly increased crude protein content compared with the control. Dong et al. [24] reported that lactic acid bacteria and fermentation substrate addition in mulberry leaf silage reduced ammonia nitrogen/total nitrogen ratio and increased dry matter content. Zhang et al. [25] also found that lactic acid bacteria and cellulase addition in alfalfa silage significantly reduced ammonia nitrogen content while preserving more crude protein.

Cellulase supplementation in silage facilitates plant cell wall decomposition, reducing fiber components and improving forage nutritional value. Colombatto et al. [26] demonstrated that cellulase addition enhanced neutral detergent fiber degradation in microbial fermentation, consistent with the significantly lower NDF and ADF contents observed in the M group compared with the control. This effect may also be attributed to acid hydrolysis of cell wall components under the low pH conditions created by the additives.

Conclusion

This study demonstrated that adding molasses, enzyme-bacteria composite preparation, and preservatives to hybrid paper mulberry silage significantly improved both fermentation quality and nutritional value. The MTM and FFJ groups maintained lower pH and better preserved crude protein and dry matter, with the FFJ group achieving the best results. From an economic perspective, adding molasses + enzyme-bacteria composite preparation (MTM group) also represents a viable approach for producing high-quality hybrid paper mulberry silage.

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Note: Figure translations are in progress. See original paper for figures.