Effects of Replacing Alfalfa Hay with Ramie Silage on Production Performance, Milk Composition, and Serum Indices in Dairy Cows: Postprint

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Abstract

This experiment aimed to investigate the effects of replacing alfalfa hay with ramie silage in diets on production performance, milk composition, and serum indices of dairy cows. Twenty-four healthy Holstein dairy cows with similar body weight, parity, and milk yield were selected and randomly allocated to 4 groups with 6 cows per group. Each group had alfalfa hay in the basal diet replaced with ramie silage at 0 (control group), 33% (Trial Group 1), 67% (Trial Group 2), and 100% (Trial Group 3), respectively. The preliminary period lasted 14 days, and the formal experimental period lasted 60 days. The results showed: 1) The contents of crude protein, crude fat, crude fiber, and crude ash in ramie silage were all higher than those in alfalfa hay. 2) Replacing alfalfa hay with ramie silage had no significant effects on dry matter intake, milk yield, or milk composition of dairy cows ($P>0.05$). 3) Replacing alfalfa hay with ramie silage had no significant effects on serum total protein, albumin, glucose, triglyceride, or glutathione peroxidase contents, nor on aspartate aminotransferase or alanine aminotransferase activities ($P>0.05$). 4) The serum urea nitrogen content in Trial Group 3 was significantly higher than that in Trial Group 1 and Trial Group 2 ($P<0.05$), but showed no significant difference from the control group ($P>0.05$). 5) The serum total cholesterol content in the control group and Trial Group 1 was significantly higher than that in Trial Group 2 ($P<0.05$), with no significant difference between Trial Group 2 and Trial Group 3 ($P>0.05$). 6) With increasing proportions of ramie silage supplementation, serum total antioxidant capacity and superoxide dismutase content increased gradually, with Trial Group 3 being significantly higher than the control group ($P<0.05$). In conclusion, ramie silage can replace alfalfa hay in dairy cow diets without affecting production performance, milk composition, or serum indices, with a suitable

replacement proportion of 33%~67%.

Full Text

Effects of Replacing Alfalfa Hay with Ramie Silage on Production Performance, Milk Composition and Serum Parameters of Dairy Cows

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Abstract

This experiment was conducted to investigate the effects of replacing alfalfa hay with ramie silage in the diet on production performance, milk composition, and serum parameters of dairy cows. Twenty-four healthy Holstein cows with similar body weight, parity, and milk yield were randomly divided into four groups (n=6). The four groups received diets where 0% (control), 33% (Trial Group 1), 67% (Trial Group 2), and 100% (Trial Group 3) of alfalfa hay was replaced with equivalent amounts of ramie silage. The pre-trial period lasted 14 days, followed by a 60-day experimental period. The results showed that: (1) Ramie silage contained higher levels of crude protein, ether extract, crude fiber, and crude ash compared to alfalfa hay. (2) Replacement of alfalfa hay with ramie silage had no significant effects on dry matter intake, milk yield, or milk composition ($P>0.05$). (3) Serum total protein, albumin, glucose, triglyceride, and glutathione peroxidase levels, as well as aspartate aminotransferase and alanine aminotransferase activities, were not significantly affected by the replacement ($P>0.05$). (4) Serum urea nitrogen content in Trial Group 3 was significantly higher than in Trial Groups 1 and 2 ($P<0.05$), but did not differ significantly from the control group ($P>0.05$). (5) Serum total cholesterol content in the control group and Trial Group 1 was significantly higher than in Trial Group 2 ($P<0.05$), with no significant difference between Trial Groups 2 and 3 ($P>0.05$). (6) With increasing proportions of ramie silage, serum total antioxidant capacity and superoxide dismutase content gradually increased, with Trial Group 3 showing significantly higher values than the control group ($P<0.05$). In conclusion, ramie silage can replace alfalfa hay in dairy cow diets without adversely affecting production performance, milk composition, or serum parameters, with an optimal replacement proportion of 33% to 67%.

Keywords: ramie silage; Holstein cow; production performance; milk composition; serum biochemical parameter; serum antioxidant parameter

Introduction

China is a major agricultural nation where agriculture forms the foundation of the national economy. The Ministry of Agriculture' s *National Plan for Herbivorous Livestock Development (2016-2020)* emphasized the need to actively develop dairy production in southern production regions and promote forage production in southern areas according to local conditions. Similarly, the *National Plan for Crop Planting Structure Adjustment (2016-2020)* specified that northern China should focus on developing high-quality alfalfa, corn silage, and forage oats, while southern regions should prioritize forage rapeseed, forage ramie, and forage mulberry.

Ramie (*Boehmeria nivea*) exhibits strong ecological adaptability, growing normally in southern China while producing high biomass. With its high crude protein content and complete amino acid profile, ramie represents a potential high-quality unconventional protein forage. Comparative studies have shown that ramie contains over 18% crude protein, significantly higher than other forages widely cultivated in southern China such as *Pennisetum purpureum* cv. Guimu-1 and perennial ryegrass. Research on feeding ramie to Landes geese demonstrated that diets containing up to 50% ramie, supplemented with concentrate feed, did not negatively affect growth performance while improving feeding efficiency. In laying hens, diets containing 6% ramie leaf meal maintained comparable egg production and culling rates while significantly reducing mortality.

Alfalfa is one of the world' s most important high-quality forages, rich in plant protein. Its carbohydrates are fermented by rumen microorganisms to produce volatile fatty acids, which serve as the primary energy source for ruminants. However, alfalfa thrives in warm, semi-arid climates with optimal daily temperatures of 15–21°C and is intolerant of waterlogged soils. Consequently, the hot, humid, and rainy conditions in southern China are unfavorable for alfalfa growth. With China' s dairy industry developing rapidly and demand for alfalfa increasing annually, domestic production cannot meet market needs, necessitating substantial imports. The shortage of high-quality forage in southern China has become a bottleneck restricting local pastoral development. Developing new high-quality forage resources is crucial for alleviating competition between humans and livestock for grain resources and promoting sustainable green development of animal husbandry in southern China. Traditional haymaking methods are limited by the hot, rainy seasons in the south, and ramie grows rapidly, becoming lignified if not harvested promptly. Silage production offers an excellent solution to these challenges. Currently, research on the scientific and efficient utilization of ramie silage in animal production is limited, and its application in dairy production is particularly scarce. This study investigated the effects of replacing alfalfa hay with ramie silage on dairy cow performance, milk com-

position, and serum parameters to provide a theoretical basis for developing ramie applications in dairy production and promoting pastoral development in southern China.

Materials and Methods

1.1 Experimental Material

Ramie silage was prepared by harvesting fresh ramie plants at approximately 1.5 m height, chopping the whole plant to approximately 3 cm length using a rubbing cutter, then baling and wrapping with a silage baler for sealed preservation.

1.2 Experimental Animals and Management

Twenty-four healthy Holstein cows with similar body weight, parity, and milk yield were randomly allocated to four groups (n=6). All experimental cows were housed in the same barn and fed total mixed rations (TMR) formulated for high utilization efficiency. Feed was provided twice daily at 05:30 and 17:30, with free access to water and loose housing management. Cows were milked twice daily in the milking parlor (05:30–06:00 and 17:30–18:00). The experimental period lasted 74 days, including a 14-day pre-trial period and a 60-day formal trial period.

1.3 Experimental Diets

Diets were formulated according to NRC (2001) dairy cattle nutrient requirements. The control group received a basal diet, while treatment groups had 33% (Trial Group 1), 67% (Trial Group 2), and 100% (Trial Group 3) of alfalfa hay in the basal diet replaced with equivalent amounts of ramie silage. Diet composition and nutrient levels are presented in Table 1 .

1.4 Sample Collection and Analysis

1.4.1 Feed Nutrient Composition After silage maturation, samples of ramie silage and alfalfa hay were collected for nutrient analysis. Samples were dried at 65°C to produce air-dried samples, which were then ground through a 40-mesh sieve. Conventional nutrient analysis was performed according to Zhang Liying (2007).

1.4.2 Feed Intake, Milk Yield, and Milk Composition Dry matter intake (DMI) was calculated based on daily feed offered and refused, along with their DM content. Milk yield was recorded daily throughout the trial period. On day 60 of the formal trial, milk samples were collected proportionally to morning and evening yields, with 50 mL total collected per cow and preserved with potassium dichromate (0.6 mg/mL) at 4°C for analysis. Milk composition was determined by Hunan Deren Animal Husbandry Co., Ltd.

1.4.3 Serum Parameters Blood samples (10 mL) were collected from the tail vein before morning feeding on day 60 using vacuum tubes, centrifuged at 2,000×g for 10 minutes, and the serum was stored at -20°C for analysis. Serum biochemical parameters including glucose, total protein, albumin, triglycerides, total cholesterol, urea nitrogen, and activities of alanine aminotransferase and aspartate aminotransferase were measured by the Hunan Drug Safety Evaluation Research Center. Serum antioxidant parameters (total antioxidant capacity, superoxide dismutase, and glutathione peroxidase) were determined using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.4.4 Statistical Analysis Data were processed using Microsoft Excel 2013. One-way ANOVA was performed using SPSS 21.0 software, and Duncan' s multiple comparison test was used for inter-group difference analysis. Significance was set at $P<0.05$, and results are expressed as means ± standard error.

Results

2.1 Nutrient Composition of Ramie Silage and Alfalfa Hay

Upon opening the silage bales, sensory evaluation revealed that ramie silage was soft in texture with clearly visible stem and leaf structure, aromatic odor, and light brown color after drying. Compared with alfalfa hay, ramie silage contained higher levels of crude protein, ether extract, crude fiber, and crude ash (Table 2).

2.2 Effects of Ramie Silage on Production Performance

As shown in Table 3 , replacing alfalfa hay with ramie silage had no significant effects on DMI, milk yield, or milk composition ($P>0.05$). However, increasing ramie silage proportion tended to decrease DMI ($P=0.567$) while milk yield showed an increasing trend ($P=0.820$) and milk somatic cell count exhibited a decreasing trend ($P=0.633$).

2.3 Effects of Ramie Silage on Serum Parameters

Table 4 shows that ramie silage replacement had no significant effects on serum total protein, albumin, glucose, triglyceride, or glutathione peroxidase levels, nor on aspartate aminotransferase or alanine aminotransferase activities ($P>0.05$). Serum urea nitrogen in Trial Group 3 was significantly higher than in Trial Groups 1 and 2 ($P<0.05$) but did not differ from the control group ($P>0.05$). Serum total cholesterol in the control group and Trial Group 1 was significantly higher than in Trial Group 2 ($P<0.05$), with no significant difference between Trial Groups 2 and 3 ($P>0.05$). With increasing ramie silage proportion, serum total antioxidant capacity and superoxide dismutase content increased, with Trial Group 3 showing significantly higher values than the control group ($P<0.05$).

Discussion

3.1 Comparison of Nutrient Composition Between Ramie Silage and Alfalfa Hay

Alfalfa is a perennial legume forage with high protein content, good palatability, and abundant amino acids with a composition similar to animal protein, which can improve dairy production performance and milk quality. Alfalfa typically contains 16.00–26.00% crude protein and 17.20–40.60% crude fiber. Ramie serves not only as a fiber crop but also as a high-protein quality forage that can be harvested multiple times annually, yielding high biomass. Studies have reported ramie crude protein content of 17.90–21.11% and crude fiber content of 7.74–26.47%. Southern China' s dairy industry lags behind the north due to natural conditions such as high temperature and humidity, but also critically due to the shortage of high-quality roughages like alfalfa and *Leymus chinensis*, and the unbalanced annual supply of roughage. Therefore, developing and screening high-quality protein forage resources in southern China will provide a solid material foundation for regional pastoral development.

3.2 Effects of Ramie Silage on Dairy Production Performance

Due to geographical and climatic limitations, alfalfa cannot meet the demands of the livestock industry in southern China, making exploration of alternative forages essential. Studies investigating the replacement of alfalfa hay or silage with fungal residue or tall fescue hay found that fungal residue could partially replace alfalfa hay without adverse effects on production performance or liver function, while tall fescue replacement decreased DMI but did not affect milk yield or composition. De Toledo et al. (2008) observed that replacing 15% of alfalfa hay with ramie in rabbit diets showed good synergistic effects and improved growth performance. In the present study, the tendency for decreased DMI with increasing ramie silage proportion may be attributed to the higher crude fiber content in ramie. We also observed a trend toward reduced milk somatic cell count, though not statistically significant. Ramie roots and leaves have medicinal properties; the leaves are sweet, cold in nature, non-toxic, and possess anti-inflammatory and blood-activating effects. Under our experimental conditions, replacing alfalfa hay with ramie silage had no significant effects on milk yield or composition, suggesting that ramie silage can partially or completely replace alfalfa hay in the short term, though long-term effects require further investigation.

3.3 Effects of Ramie Silage on Serum Parameters

Serum urea nitrogen content correlates with crude protein intake, rumen degradable protein utilization by microorganisms, and protein utilization efficiency in the body. When protein utilization decreases, serum urea nitrogen increases, whereas low levels indicate higher protein synthesis efficiency. In this study, the significantly higher serum urea nitrogen in Trial Group 3 compared to Trial

Groups 1 and 2 may indicate lower protein synthesis efficiency, while the lower values in Trial Groups 1 and 2 suggest complementary and synergistic effects between ramie silage and alfalfa hay that improve protein utilization. Ramie leaves are rich in organic acids, aldehydes, ketones, esters, and sterols, with organic acids being the predominant compounds. The unsaturated fatty acid content exceeds 45%, and unsaturated fatty acids help maintain normal cellular physiological function and reduce blood cholesterol and triglyceride levels. Our findings demonstrate that adding ramie silage to dairy diets at certain levels significantly reduced serum total cholesterol content. Moreover, ramie silage significantly enhanced serum total antioxidant capacity and superoxide dismutase content, indicating its potential to alleviate oxidative stress in animals.

Based on these results, we conclude that: (1) Ramie silage has balanced nutritional value, particularly its high crude protein content, and can replace alfalfa hay without significantly affecting milk yield or composition, while reducing serum cholesterol and enhancing antioxidant capacity; and (2) The optimal replacement proportion of alfalfa hay with ramie silage in dairy cow diets is 33% to 67%.

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