Effects of Partial Replacement of Alfalfa Hay with Fresh Alfalfa on Production Performance and Serum Biochemical Parameters in Dairy Cows: Postprint

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Abstract

This experiment aimed to investigate the effects of replacing different proportions of alfalfa hay with fresh alfalfa on production performance, digestion and metabolism, and serum biochemical indices in dairy cows. Eighteen healthy Holstein dairy cows in mid-lactation with similar body weight, parity, and milk yield were selected and randomly divided into 3 groups with 6 cows per group. The control group was fed a basal diet, while experimental group I and experimental group II were fed experimental diets in which 50% and 75% of alfalfa hay in the basal diet were replaced with fresh alfalfa, respectively. The preliminary feeding period lasted 15 days, and the formal experimental period lasted 30 days. The results showed that: 1) Replacing different proportions of alfalfa hay with fresh alfalfa had no significant effects on dry matter intake and milk yield in dairy cows ($P>0.05$), but milk somatic cell counts in all experimental groups were significantly lower than that in the control group ($P<0.05$). 2) Replacing different proportions of alfalfa hay with fresh alfalfa had no significant effect on the apparent digestibility of nutrients in dairy cows ($P>0.05$). 3) Replacing different proportions of alfalfa hay with fresh alfalfa had no significant effects on the contents of total protein, albumin, globulin, cholesterol, glucose, triglycerides, high-density lipoprotein, and low-density lipoprotein, as well as the activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in serum of dairy cows ($P>0.05$). The serum urea nitrogen content in experimental group I was significantly higher than that in the control group and experimental group II ($P<0.05$), with no significant difference between the control group and experimental group II ($P>0.05$). 4) Compared with the control group, the economic benefits of both experimental group I and experimental group II increased, with experimental group II having the

highest net profit increase at 9.06 yuan/(head · d). In conclusion, fresh alfalfa can partially replace alfalfa hay in dairy cow diets without affecting production performance, digestion and metabolism, and serum biochemical indices, while increasing economic benefits.

Full Text

Effects of Partial Replacement of Alfalfa Hay with Fresh Alfalfa on Performance and Serum Biochemical Parameters of Dairy Cows

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Abstract

This experiment was conducted to investigate the effects of replacing different proportions of alfalfa hay with fresh alfalfa on production performance, nutrient digestibility, and serum biochemical parameters in dairy cows. Eighteen healthy Holstein cows in mid-lactation with similar body weight, parity, and milk yield were randomly allocated into three groups of six cows each. The control group was fed a basal diet, while trial groups I and II were fed experimental diets in which 50% and 75% of the alfalfa hay in the basal diet was replaced with fresh alfalfa, respectively. The experiment consisted of a 15-day preliminary period followed by a 30-day formal trial period. The results showed that: (1) Replacement of alfalfa hay with different proportions of fresh alfalfa had no significant effect on dry matter intake or milk yield ($P>0.05$), but milk somatic cell count in both trial groups was significantly lower than in the control group ($P<0.05$). (2) Apparent digestibility of nutrients was not significantly affected by the replacement ($P>0.05$). (3) Serum total protein, albumin, globulin, cholesterol, glucose, triglyceride, high-density lipoprotein, and low-density lipoprotein concentrations, as well as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities were not significantly affected by the replacement ($P>0.05$). Serum urea nitrogen content in trial group I was significantly higher than in the control group and trial group II ($P<0.05$), with

no significant difference between the control group and trial group II (P>0.05). (4) Compared with the control group, the economic benefit increased in both trial groups I and II, with trial group II showing the highest added profit of 9.06 yuan/(head · d). In conclusion, fresh alfalfa can partially replace alfalfa hay in dairy cow diets without affecting production performance, nutrient digestibility, or serum biochemical parameters, while improving economic benefits.

Keywords: dairy cows; fresh alfalfa; alfalfa hay; production performance; serum biochemical parameters

Introduction

Global dairy production has developed rapidly in recent years. According to 2016 data from the Food and Agriculture Organization of the United Nations, global raw milk production reached 817 million tons, representing a 1.1% increase from 2015 [1]. Over the past decade, China' s dairy industry has also embarked on a new journey, with the government attaching great importance to dairy production and actively promoting policies for dairy revitalization such as "growing good grass, raising good cows, and producing good milk" [2]. As agricultural industrial structure undergoes deep adjustment and the livestock industry shifts from grain-consuming to grain-saving models, "growing good grass" has become particularly important in livestock production in agricultural regions.

Alfalfa (*Medicago sativa*), recognized worldwide as a high-quality leguminous forage, typically contains 16.00%–26.00% crude protein and 17.20%–40.60% crude fiber [3]. As a premium feed for cattle, sheep, pigs, chickens, geese, and fish, it can increase milk yield and daily weight gain, improve meat and egg quality, and enhance immune function [4]. In the 1990s, alfalfa cultivation was rapidly promoted and extensively planted in northern agricultural and pastoral regions of China, while development in southern regions progressed relatively slowly. In recent years, as the dairy industry has gradually developed in southern China, demand for alfalfa products has grown dramatically. Progress has been made in screening alfalfa varieties suitable for southern regions and researching stress resistance, and alfalfa cultivation and production in the south has begun to take shape [5]. However, due to the humid climate in southern regions, producing hay is difficult. Therefore, exploring forage processing and utilization models suitable for the characteristics of southern regions such as Hunan has important practical significance and promotional value.

Previous research has extensively investigated various utilization modes of alfalfa. Currently, alfalfa is primarily used as hay in livestock production, with some utilization as silage, alfalfa meal, or extraction of bioactive compounds. For example, Shi et al. [6] reported that feeding alfalfa silage to dairy cows increased dry matter intake and milk yield. Fan et al. [7] and Zhou et al. [8] added alfalfa saponins to laying hen diets to improve production performance.

Zhang et al. [9] added 8% alfalfa meal to carp feed to improve growth performance. Although alfalfa silage has received attention in recent years, protein degradation during the ensiling process affects protein utilization efficiency [10], and the production process requires substantial labor and material resources. During hay preparation, losses from rain and leaf drop typically reach approximately 30% [11-12], causing nutrient loss, particularly of protein, and resulting in resource waste [13-14]. Ultimately, the goal of exploring different utilization methods is to improve effective forage utilization efficiency. For the dairy industry in southern China, alfalfa hay supply still primarily relies on northern regions or imports. High transportation and procurement costs have led to persistently high production costs in southern regions, which is detrimental to dairy farming operations [15-16]. Although alfalfa cultivation techniques in southern regions have matured, traditional hay preparation methods are easily constrained by the hot and humid climate. Therefore, exploring the direct feeding of fresh alfalfa to dairy cows may be an effective approach to address the shortage of high-quality roughage in southern dairy farming. Currently, relevant research on feeding fresh alfalfa to dairy cows is scarce. This experiment aimed to investigate the effects of replacing different proportions of alfalfa hay with fresh alfalfa on production performance, nutrient digestibility, and blood biochemical parameters in dairy cows, providing experimental reference for rational processing and utilization of alfalfa and other forages in southern regions.

Materials and Methods

1.1 Experimental Materials

Fresh alfalfa was cultivated in Huangnihu Village, Xihu District, Changde City, Hunan Province. The fresh forage was harvested at the budding stage and chopped to approximately 3 cm length using a chaff cutter for experimental use. Fresh alfalfa was harvested and used daily. Alfalfa hay used in the experiment was imported from Idaho, USA, and stored in a dry, dark place.

1.2 Experimental Design and Animal Management

Eighteen healthy Holstein cows in mid-lactation with 2–3 parities, average body weight of (600±50) kg, and milk yield of (22.5±4.3) kg/d were selected and randomly allocated into three groups of six cows each using a single-factor randomized block design. Experimental cows were housed in the same barn and fed in groups. All groups were fed total mixed rations (TMR) twice daily at 07:00 and 18:00 in equal amounts, with daily refusals controlled at 5%–10%. Cows had free access to water throughout the experimental period. Milking was performed twice daily (06:30–07:00 and 17:30–18:00) using an automatic pipeline milking machine. After milking, cows were moved to an exercise yard for free activity. The barn was cleaned twice daily and thoroughly disinfected once weekly to maintain hygiene. The experimental period lasted 45 days, including

a 15-day preliminary period and a 30-day formal trial period.

1.3 Experimental Diets

Experimental diets were formulated according to NRC (2001) dairy nutrition requirements, with a concentrate-to-forage ratio of 45:55 (dry matter basis). The control group was fed a basal diet consisting of concentrate, corn silage, oat grass, beet pulp, and alfalfa hay. Trial groups I and II were fed experimental diets in which 50% and 75% of the alfalfa hay in the basal diet was replaced with fresh alfalfa, respectively. The composition and nutrient levels of experimental diets are shown in Table 1 .

Table 1 Composition and nutrient levels of experimental diets

Note: The premix provided the following per kg of diet: VA 550,000 IU, VD 120,000 IU, VE 2,100 IU, Cu 1,377 mg, Fe 1,400 mg, Zn 6,300 mg, Mn 1,350 mg, Se 44 mg, I 80 mg, Co 18 mg.

1.4 Measurements

1.4.1 Nutrient Content Determination Fresh alfalfa was harvested at the budding stage, and samples of both fresh alfalfa and alfalfa hay were collected for nutrient content analysis. Samples were dried at 65°C to produce air-dried samples, which were then ground to pass through a 40-mesh sieve. Dry matter, crude protein, crude fat, and crude ash contents were determined using conventional methods described by Yang [17]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using a Fibretherm FT12 automatic fiber analyzer (Gerhardt Analytical Systems, Germany) according to the method of Hall et al. [18].

1.4.2 Feed Intake, Milk Yield, and Milk Composition Dry matter intake was calculated based on daily feed offered, refusals, and their dry matter content for each cow. Milk yield was recorded daily throughout the trial period. On day 30 of the formal trial, milk samples were collected proportionally to morning and evening yields using a sampling cup, with a total of 50 mL collected per cow. Potassium dichromate preservative (0.6 mg/mL) was added, mixed thoroughly, and stored at 4°C for analysis. Milk composition was analyzed using a Milko-Scan 134A/B automatic milk composition analyzer.

1.4.3 Apparent Nutrient Digestibility A digestion trial was conducted from days 24–30 of the formal trial period. Approximately 300 g of fresh feces was collected from each cow 30 minutes before morning and evening feeding. Fecal samples collected over 7 consecutive days were thoroughly mixed, and composite samples were prepared as follows: (1) 100 g of fresh feces was mixed with 10 mL of 10% sulfuric acid, dried at 65°C for 48 hours, and ground to pass through a 40-mesh sieve for crude protein determination. (2) Approximately 200 g of fresh feces was dried at 65°C for 48 hours, initial moisture was determined,

and samples were ground to pass through a 40-mesh sieve for other nutrient determinations. Nutrient content in fecal samples was determined using the methods described in section 1.4.1.

Apparent nutrient digestibility was determined using the 4-N hydrochloric acid insoluble ash method. Hydrochloric acid insoluble ash content in fecal samples was determined using the incineration method according to "Determination of Hydrochloric Acid Insoluble Ash in Feed" (GB/T 23742–2009).

1.4.4 Serum Biochemical Parameters On day 30 of the formal trial, blood samples (10 mL per cow) were collected from the tail vein 30 minutes before morning feeding using vacuum tubes. Samples were centrifuged at 2,000×g for 10 minutes, and serum was harvested and stored at -20°C for analysis. Serum total protein, albumin, globulin, cholesterol, glucose, urea nitrogen, triglyceride, high-density lipoprotein, and low-density lipoprotein concentrations, as well as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities were determined using an automatic biochemical analyzer (Hitachi 7020, Japan).

1.5 Statistical Analysis

Data were initially processed using Excel 2013 and analyzed using SPSS 21.0 software. One-way ANOVA was performed, and Duncan' s multiple comparison test was used to detect significant differences among groups. Significance was defined as $P<0.05$. Results are expressed as mean ± standard deviation.

Results

2.1 Nutrient Composition of Fresh Alfalfa and Alfalfa Hay

The conventional nutrient composition of fresh alfalfa cultivated in southern China and imported alfalfa hay is shown in Table 2 . The data indicate that fresh alfalfa had slightly higher crude protein, crude fat, and crude ash contents, and lower neutral detergent fiber and acid detergent fiber contents compared with alfalfa hay.

Table 2 Common nutrient component contents of fresh alfalfa and alfalfa hay (DM basis)

2.2 Effects on Dry Matter Intake, Milk Yield, and Milk Composition

As shown in Table 3 , replacing alfalfa hay with different proportions of fresh alfalfa had no significant effect on dry matter intake or milk yield ($P>0.05$), and no significant effects on milk protein percentage, milk fat percentage, lactose percentage, milk urea nitrogen percentage, or milk dry matter percentage ($P>0.05$). However, milk somatic cell count in both trial groups I and II was

significantly lower than in the control group (P<0.05), with no significant difference between the two trial groups (P>0.05). As the replacement proportion of fresh alfalfa increased, milk yield showed a gradual increasing trend.

Table 3 Effects of replacing alfalfa hay with different proportions of fresh alfalfa on DMI, milk yield, and milk composition of dairy cows

Note: Values in the same row with different small letter superscripts indicate significant difference (P<0.05). The same applies below.

2.3 Effects on Apparent Nutrient Digestibility

As shown in Table 4 , replacing alfalfa hay with different proportions of fresh alfalfa had no significant effect on apparent digestibility of crude protein, neutral detergent fiber, or acid detergent fiber (P>0.05).

Table 4 Effects of replacing alfalfa hay with different proportions of fresh alfalfa on nutrient apparent digestibility of dairy cows

2.4 Effects on Serum Biochemical Parameters

As shown in Table 5 , replacing alfalfa hay with different proportions of fresh alfalfa had no significant effect on serum total protein, albumin, globulin, cholesterol, glucose, triglyceride, high-density lipoprotein, or low-density lipoprotein concentrations, nor on alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase activities (P>0.05). Serum urea nitrogen content in trial group I was significantly higher than in the control group and trial group II (P<0.05), with no significant difference between the control group and trial group II (P>0.05).

Table 5 Effects of replacing alfalfa hay with different proportions of fresh alfalfa on serum biochemical parameters in dairy cows

2.5 Effects on Economic Benefit

The cost of alfalfa in the experimental diets was 44.60 yuan/(head · d). The prices of alfalfa hay and fresh alfalfa were 2.60 and 0.40 yuan/kg, respectively. The uniform purchase price for qualified milk during the trial period was 4.50 yuan/kg. Economic calculations (Table 6) showed that feed costs in trial groups I and II were reduced by 5.04 and 7.20 yuan/(head · d), respectively, while added profits were 6.30 and 9.06 yuan/(head · d), respectively.

Table 6 Effects of replacing alfalfa hay with different proportions of fresh alfalfa on economic benefits in dairy cows (yuan/(head · d))

Discussion

3.1 Effects on Dairy Cow Performance

Alfalfa is a perennial high-quality leguminous forage with high protein content and abundant amino acids that can improve dairy cow performance and milk quality, and is widely used in dairy production [19-20]. For the dairy industry in southern China, the current shortage of high-quality roughage urgently requires regionally appropriate development of forage and animal husbandry. The high procurement and transportation costs of imported high-quality hay have become a major factor restricting the development of animal husbandry in southern regions. Domestic research has investigated the replacement of alfalfa hay with alfalfa silage or ramie silage. Zhu et al. [21] reported that replacing 2.0 kg of alfalfa hay (dry matter basis) with 4.4 kg of alfalfa silage had no significant effect on dry matter intake or milk yield, but improved milk protein and fat percentages, thereby increasing production benefits. Well-prepared alfalfa silage maintains the nutritional composition of fresh alfalfa while offering advantages such as high digestibility, good palatability, and storability [22-23]. Direct feeding of fresh alfalfa completely preserves its nutritional composition and, compared with alfalfa silage, reduces the loss of soluble protein and prevents inadequate absorbable protein intake by cows [24]. Additionally, as a green forage with more comprehensive nutrition, fresh alfalfa may enhance cow immunity, thereby reducing milk somatic cell count [25]. This experiment found that as the replacement proportion of fresh alfalfa increased, milk yield showed an increasing trend and milk somatic cell count decreased significantly, indicating that fresh alfalfa can promote cow health, particularly mammary gland health. Under the conditions of this experiment, replacing 50% or 75% of alfalfa hay in dairy cow diets with fresh alfalfa had no significant effect on production performance indicators such as milk yield and composition, preliminarily demonstrating that fresh alfalfa can replace alfalfa hay in the short term. However, the long-term effects of replacement require further investigation.

3.2 Effects on Nutrient Apparent Digestibility

Fecal excretion loss rate is a comprehensive reflection of dietary nutrient digestibility [26]. As a green forage, alfalfa is rich in vitamins that can promote nutrient digestion and absorption in animals. Its alkaline chemical properties aid diet digestion, stimulate gastrointestinal motility, and have a laxative effect [27]. Peng et al. [28] reported that appropriate replacement of concentrate with fresh alfalfa in gestating sow diets improved apparent digestibility of crude protein, neutral detergent fiber, and acid detergent fiber, improved sow body condition, and shortened non-productive days. Kang et al. [29] found that nitrogen apparent metabolism rate in dairy cows fed alfalfa silage was significantly higher than in those fed alfalfa hay. This study found no significant differences among groups in apparent digestibility of crude protein, neutral detergent fiber, or acid detergent fiber. Although fresh alfalfa had slightly higher crude protein content than alfalfa hay, and fresh alfalfa contains more abundant bioactive

compounds, is more succulent, and has better palatability and flexibility, these factors were insufficient to affect apparent nutrient digestibility.

3.3 Effects on Serum Biochemical Parameters

Serum biochemical parameters reflect animal metabolism and health status. Serum urea nitrogen and total protein concentrations are two important indicators of protein metabolism, with serum urea nitrogen more accurately reflecting protein metabolism in animals. When protein utilization decreases, serum urea nitrogen content increases. Lower serum urea nitrogen content indicates enhanced protein synthesis, increased nitrogen deposition, and consequently increased total protein concentration in blood [30]. In this experiment, serum urea nitrogen content in trial group I was significantly higher than in trial group II and the control group, possibly because the 50% replacement level in trial group I affected protein synthesis to some extent, and the mixture of 50% fresh alfalfa and 50% alfalfa hay was not conducive to protein absorption and utilization. The lower serum urea nitrogen content in trial group II preliminarily indicates higher protein utilization at the 75% replacement level. Serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities, along with cholesterol concentration, are important indicators for detecting abnormal liver metabolism [31-32]. In this experiment, these indicators in all groups were within normal ranges and showed no significant differences among groups, indicating that fresh alfalfa had no adverse effects on liver metabolism in dairy cows and further demonstrating that partial replacement of alfalfa hay with fresh alfalfa is feasible.

Conclusion

Under the conditions of this experiment, replacing 50% and 75% of alfalfa hay in the basal diet with fresh alfalfa had no significant effect on dry matter intake, milk yield, milk fat percentage, or milk protein percentage in dairy cows, but reduced milk somatic cell count and feed costs while improving economic benefits.

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