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 study 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 mid-lactation Holstein dairy cows with similar body weight, parity, and milk yield were randomly allocated into three groups (n=6 per group). The control group was fed a basal diet, while experimental groups I and II were fed experimental diets in which 50% and 75% of the alfalfa hay in the basal diet were replaced by fresh alfalfa, respectively. The experiment consisted of a 15-day preliminary period followed by a 30-day formal experimental period. The results showed: 1) Replacement of alfalfa hay with fresh alfalfa at different proportions had no significant effect on dry matter intake or milk yield ($P>0.05$), but milk somatic cell counts in all experimental groups were significantly lower than that in the control group ($P<0.05$). 2) No significant effects were observed on apparent nutrient digestibility ($P>0.05$). 3) Serum contents of total protein, albumin, globulin, cholesterol, glucose, triglycerides, high-density lipoprotein, and low-density lipoprotein, as well as activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were not significantly affected ($P>0.05$). However, serum urea nitrogen content in experimental group I was significantly higher than that in both the control group and experimental group II ($P<0.05$), with no significant difference between the latter two groups ($P>0.05$). 4) Economic benefits increased in both experimental groups compared with the control group, with experimental group II achieving the highest net profit increase of 9.06 yuan/(head · d). It is concluded that fresh alfalfa can partially replace alfalfa hay in dairy cow diets without compromising production performance, digestion and metabolism, or

serum biochemical indices, while improving economic returns.

Full Text

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Abstract

This experiment aimed to investigate the effects of replacing alfalfa hay with different proportions of fresh alfalfa on production performance, digestion and metabolism, and serum biochemical parameters in dairy cows. Eighteen healthy Holstein cows at mid-lactation with similar parity, body weight, and milk yield were randomly divided into three groups with six cows per group. The control group was fed a basal diet, while trial groups I and II were fed experimental diets in which 50% and 75% of alfalfa hay in the basal diet was replaced with fresh alfalfa, respectively. The pretrial period lasted for 15 days, and the formal trial period lasted for 30 days. The results showed that: (1) Replacing 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 that in the control group ($P<0.05$). (2) Nutrient apparent digestibility 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 contents, as well as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities were not significantly affected ($P>0.05$). Serum urea nitrogen content in trial group I was significantly higher than that in the control group and trial group II ($P<0.05$), but no significant difference was observed between the control group and trial group II ($P>0.05$). (4) Compared with the control group, the economic benefits of trial groups I and II both increased, 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, digestion and metabolism, or serum biochemical parameters, while increasing 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 forage, raising good cattle, 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 forage" has become particularly important in livestock production in agricultural regions.

Alfalfa (Medicago sativa) is recognized worldwide as a high-quality leguminous forage, with crude protein content generally ranging from 16.00% to 26.00% and crude fiber content from 17.20% to 40.60% [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 alfalfa variety selection and stress resistance research for southern regions, and alfalfa cultivation and production in the south have begun to take shape [5]. However, due to the humid climate in southern regions, preparing hay is difficult. Therefore, exploring forage processing and utilization models suitable for southern regions such as Hunan has important practical significance and promotional value.

Previous research has extensively investigated alfalfa utilization patterns. 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] fed alfalfa silage to dairy cows and increased their 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. Alfalfa silage has received increased attention in recent years, but protein degradation during ensiling affects protein utilization efficiency [10], and the production process requires substan-

tial labor and material investment. 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. For the dairy industry in southern regions, alfalfa hay supply still primarily depends on northern regions or imports. High transportation and procurement costs have kept production costs high 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 southern climate. Therefore, exploring the 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 provide experimental reference for rational processing and utilization of alfalfa and other forages in southern regions by investigating the effects of replacing different proportions of alfalfa hay with fresh alfalfa on dairy cow production performance, digestion and metabolism, and blood biochemical parameters.

Materials and Methods

1.1 Experimental Materials

Fresh alfalfa was cultivated in Huangnihu Village, Xihu District, Changde City, Hunan Province, and harvested at the budding stage. The fresh alfalfa was then chopped to approximately 3 cm length using a chaff cutter for later use. Fresh alfalfa was harvested and used daily during the experiment. 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 at 2-3 parity, with average body weight of (600±50) kg and milk yield of (22.5±4.3) kg/d, were selected and randomly divided into three groups using a single-factor randomized block design, with six cows per group. 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 movement. The barn was cleaned twice daily and thoroughly disinfected once weekly to maintain hygiene. The experimental period lasted 45 days, including a 15-day pretrial period and a 30-day formal trial period.

1.3 Experimental Diets

Experimental diets were formulated according to NRC (2001) dairy nutrient 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 hay, beet pulp, and alfalfa hay. Trial groups I and II were fed experimental diets in which 50% and 75% of alfalfa hay in the basal diet were replaced with fresh alfalfa, respectively. Diet composition and nutrient levels are shown in Table 1 .

1.4 Analytical Methods

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 determination. Collected 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 established 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 content was determined in diet and refusal samples. Daily dry matter intake per cow was calculated based on daily feed offered, refusals, and their dry matter content. Milk yield was recorded daily during the experimental period. On day 30 of the formal trial, milk samples were collected proportionally to morning and evening yields to obtain a total of 50 mL, preserved with potassium dichromate (0.6 mg/mL), mixed thoroughly, and refrigerated at 4°C for later analysis. Milk composition was analyzed using a Milko-Scan 134A/B automatic milk composition analyzer.

1.4.3 Nutrient Apparent Digestibility A digestion and metabolism 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 each day. Fecal samples from seven 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 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 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.

Nutrient apparent 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 later analysis. Serum total protein, albumin, globulin, cholesterol, glucose, urea nitrogen, triglyceride, high-density lipoprotein, and low-density lipoprotein contents, 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 software. Statistical analysis was performed using SPSS 21.0 software for one-way ANOVA, and Duncan' s multiple comparison test was used to examine significant differences among groups. Significance was defined as $P<0.05$, and results are expressed as mean±standard deviation.

Results

2.1 Conventional Nutrient Contents of Fresh Alfalfa and Alfalfa Hay

The conventional nutrient contents of fresh alfalfa cultivated in southern regions and imported alfalfa hay are shown in Table 2 . The data indicate that fresh alfalfa had slightly higher crude protein, crude fat, and crude ash contents than alfalfa hay, while neutral detergent fiber and acid detergent fiber contents were lower than those in alfalfa hay.

2.2 Effects of Replacing Alfalfa Hay with Different Proportions of Fresh Alfalfa 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 were observed 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 that in the control group ($P<0.05$), though no significant difference was found between trial groups I and II ($P>0.05$). With increasing replacement proportion of fresh alfalfa, milk yield showed a gradual increasing trend.

2.3 Effects of Replacing Alfalfa Hay with Different Proportions of Fresh Alfalfa on Nutrient Apparent 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 ($P>0.05$).

2.4 Effects of Replacing Alfalfa Hay with Different Proportions of Fresh Alfalfa 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 contents, 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 that in the control group and trial group II ($P<0.05$), but no significant difference was observed between the control group and trial group II ($P>0.05$).

2.5 Effects of Replacing Alfalfa Hay with Different Proportions of Fresh Alfalfa on Economic Benefits

The cost of alfalfa in experimental diets was 44.60 yuan/(head · d), with prices of alfalfa hay and fresh alfalfa at 2.60 yuan/kg and 0.40 yuan/kg, respectively. Qualified milk was purchased at a uniform price of 4.50 yuan/kg during the trial period. Economic benefits calculated from these data (Table 6) showed that feed costs in trial groups I and II using fresh alfalfa decreased by 5.04 and 7.20 yuan/(head · d), respectively, while added profits were 6.30 and 9.06 yuan/(head · d), respectively.

Discussion

3.1 Effects of Partial Replacement of Alfalfa Hay with Fresh Alfalfa 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 regions, the current shortage of high-quality roughage urgently requires the development of forage and livestock industries adapted to local conditions. The high cost of purchasing and transporting expensive imported high-quality hay has become a major factor restricting the development of animal husbandry in southern regions. Domestic research has been conducted on replacing alfalfa hay with alfalfa silage or ramie silage in dairy cow diets. 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 in dairy cows, but increased milk protein and fat percentages, thereby improving farming benefits.

Well-prepared alfalfa silage maintains the nutritional composition of fresh alfalfa while offering advantages such as high digestibility, good palatability, and storage durability [22-23]. Feeding fresh alfalfa directly to dairy cows completely preserves its nutritional composition, and compared with alfalfa silage, reduces the loss of soluble protein and prevents inadequate absorbable protein intake [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 with increasing replacement proportion of fresh alfalfa, 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 on dairy cow performance require further investigation.

3.2 Effects of Partial Replacement of Alfalfa Hay with Fresh Alfalfa 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 act as a laxative [27]. Peng et al. [28] reported that appropriate replacement of concentrate with fresh alfalfa in sow diets during gestation 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 that in cows 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 compared with alfalfa hay, fresh alfalfa had more abundant bioactive substances with better palatability and flexibility, these factors may not have been sufficient to affect nutrient apparent digestibility.

3.3 Effects of Partial Replacement of Alfalfa Hay with Fresh Alfalfa on Serum Biochemical Parameters

Serum biochemical parameters can reflect animal metabolism and health status. Serum urea nitrogen and total protein contents are two important indicators reflecting protein metabolism, with serum urea nitrogen content more accurately reflecting protein metabolism in animals. When protein utilization decreases, serum urea nitrogen content increases. When serum urea nitrogen content is low, it indicates enhanced protein synthesis, increased nitrogen deposition, and

ultimately increased total protein content in blood [30]. In this experiment, serum urea nitrogen content in trial group I was significantly higher than that 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 relatively low 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 content, are important indicators for detecting abnormal liver metabolism [31-32]. In this experiment, these indicators in all groups were within normal ranges with 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 increasing economic benefits.

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