Effects of Roughage Combination on Nitrogen Utilization in Dairy Cows under Heat Stress: Postprint

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Abstract

To investigate the effects of roughage combination on nitrogen utilization in heat-stressed dairy cows, twenty healthy Holstein cows with similar parity and days in milk were selected and randomly divided into two groups (n=10 per group), which were fed two different roughage combination diets: corn silage + alfalfa (0.92:1.00, AF group) and corn stover (CS group). The concentrate was identical for both groups, with a concentrate-to-forage ratio of 63.7:36.3. The preliminary period lasted for 1 week, followed by an 8-week experimental period. The results showed that: 1) Compared with the CS group, milk yield and the yields of energy-corrected milk, 4% fat-corrected milk, milk protein, and lactose in the AF group were significantly or extremely significantly increased ($P<0.01$ or $P<0.05$). 2) The apparent digestibility of dietary crude protein in cows was extremely significantly higher in the AF group than in the CS group ($P<0.01$). 3) Compared with the CS group, rumen microbial protein yield in the AF group was significantly increased ($P<0.05$), while urinary and blood urea nitrogen concentrations were extremely significantly decreased ($P<0.01$). 4) Compared with the CS group, nitrogen intake and milk nitrogen output in the AF group were extremely significantly increased ($P<0.01$), but nitrogen conversion efficiency was significantly decreased ($P<0.05$). These results suggest that, compared with corn stover, the combination of corn silage + alfalfa can increase dietary crude protein level, enhance apparent digestibility of crude protein in heat-stressed dairy cows, and promote the synthesis of rumen microbial protein and milk protein, but the nitrogen conversion efficiency needs to be improved.

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Full Text

Effects of Forage Combinations on Nitrogen Efficiency of Dairy Cows under Heat Stress

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Abstract

To investigate the effects of forage combinations on nitrogen utilization in heat-stressed dairy cows, twenty healthy Holstein cows with similar parities and days in milk were randomly divided into two groups of ten cows each. The cows were fed two diets with different forage combinations: corn silage + alfalfa hay (0.92:1.00, AF group) and corn straw (CS group). Both groups received the same concentrate with a concentrate-to-forage ratio of 63.7:36.3. The pre-trial period lasted for 1 week, followed by an 8-week formal trial period. The results showed that: 1) Compared with the CS group, the AF group exhibited significantly or highly significantly increased milk yield, energy-corrected milk, 4% fat-corrected milk, and yields of milk protein and lactose ($P<0.01$ or $P<0.05$). 2) The apparent digestibility of crude protein (CP) in the AF group diet was highly significantly higher than that in the CS group diet ($P<0.01$). 3) Rumen microbial protein yield in the AF group was significantly higher than in the CS group ($P<0.05$), while urine and blood urea nitrogen concentrations were highly significantly lower ($P<0.01$). 4) Nitrogen intake and milk nitrogen yield in the AF group were highly significantly higher than in the CS group ($P<0.01$), but nitrogen conversion efficiency was significantly lower ($P<0.05$). These results indicate that compared with corn straw, the combination of corn silage and alfalfa hay can increase dietary CP level, improve apparent CP digestibility, and enhance microbial protein and milk protein synthesis in heat-stressed dairy cows, though nitrogen conversion efficiency remains to be improved.

Keywords: heat stress; forage; microbial protein; apparent digestibility; nitrogen efficiency

Introduction

Heat stress leads to reduced feed intake and decreased production performance in dairy cows [1]. Recent studies have found that heat stress alters nitrogen metabolism in dairy cows, reducing milk protein synthesis and causing "heat stress-induced milk protein depression syndrome" [2-4]. Additionally, the dairy industry faces challenges related to nitrogen excretion. According to Tamminga [5], only 21% of nitrogen intake is utilized by the cow, while the remaining 79% is excreted in urine and feces. Therefore, urgent measures are needed to improve nitrogen conversion efficiency and increase milk protein synthesis in heat-stressed dairy cows.

Previous studies have reported that forage type affects nitrogen digestion and utilization efficiency in dairy cows. Xia et al. [6] found that nitrogen digestibility was higher for alfalfa and Chinese wildrye than for corn straw. Ren et al. [7] also reported that forage combinations affect nitrogen conversion efficiency and milk protein synthesis. Currently, research on the effects of forage combinations on nitrogen metabolism in heat-stressed dairy cows is limited. Therefore, this study investigated the effects of forage combinations on nitrogen conversion efficiency in heat-stressed dairy cows to provide a reference for improving milk protein synthesis under heat stress conditions.

Materials and Methods

Experimental Animals and Design Based on similar days in milk [(86$±14)*d*]*andmilkyield*[(36.7±$4.3) kg/d], twenty primiparous Holstein cows were randomly divided into two groups of ten cows each. The cows were fed two diets with different forage combinations: corn silage + alfalfa hay (0.92:1.00, AF group) and corn straw (CS group). Both groups received the same concentrate with a concentrate-to-forage ratio of 63.7:36.3. The experimental diets were formulated according to NRC (2001) standards, and their composition and nutrient levels are shown in Table 1 . The pre-trial period lasted for 1 week, and the formal trial period lasted for 8 weeks. Cows were housed in an automatic feeding system (RIC system) and fed total mixed rations (TMR) twice daily (07:00 and 19:00) with free access to water. Milkings were conducted three times daily (07:00, 14:00, and 20:00).

Sample Collection and Analysis

Temperature-Humidity Index (THI) Recording and Calculation Respiratory rate and rectal temperature were measured daily at 07:00 and 14:00. Barn temperature and relative humidity were recorded at 06:00, 14:00, and 22:00 using a thermo-hygrometer. THI was calculated using the method of Cowley et al. [4] with the following formula:

$$THI = 1.8 \times T + 32 - 0.55 \times (1 - RH) \times (T \times 1.8 + 32 - 58)$$

where T is temperature (°C) and RH is relative humidity (%).

Feed and Milk Sample Collection and Analysis Daily feed intake was recorded using the automatic feeding system. Milk yield was recorded daily, and milk samples were collected continuously for 2 days each week and mixed in a ratio of morning:afternoon:evening = 4:3:3. Milk protein, fat, and lactose contents were analyzed using an automatic milk composition analyzer (Foss, Denmark) to calculate daily yields. Energy-corrected milk and 4% fat-corrected milk yields were calculated, and feed-to-milk ratio was determined based on feed intake and milk yield. Milk urea nitrogen concentration was determined according to Bhandari et al. [8]. Diet and orts samples were collected every 2 weeks, dried at 65°C, ground through a 40-mesh sieve, and stored sealed. Crude protein (CP) content was determined using an automatic Kjeldahl nitrogen analyzer (VELP UDK159, Italy). Crude fat (EE) and ash contents were determined according to AOAC (2000) [9]. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined using a fiber analyzer [Ankom (200), USA].

Feces Collection and Analysis Feces were collected using the spot sampling method on days 54, 55, and 56 of the trial. Samples were collected every 6 hours (200 g each time) and pooled into two subsamples of 500 g each after collection. One subsample was used to determine EE, ash, ADF, and NDF contents, while the other was treated with 10% 6 mol/L HCl for CP determination. CP content was determined using an automatic Kjeldahl nitrogen analyzer (VELP UDK159, Italy). EE and ash contents were determined according to AOAC (2000) [9]. ADF and NDF contents were determined using an Ankom (200) fiber analyzer (Ankom Technology, USA). Whole-tract apparent digestibility was estimated using the internal marker method with acid-insoluble ash (AIA) as the marker [10]. The calculation formula was:

$$\text{Whole-tract apparent digestibility (\%)} = 100 \times \left[1 - \frac{A_1 \times B_2}{A_2 \times B_1}\right]$$

where A_1 is the AIA content in the diet (%), A_2 is the AIA content in feces (%), B_1 is the nutrient content in the diet (%), and B_2 is the nutrient content in feces (%).

Urine Collection and Analysis Urine was collected using the spot sampling method on days 54, 55, and 56 of the trial. Samples were collected every 6 hours (30 mL each time) and pooled to 100 mL after collection. Urine was mixed with 1% 6.0 mol/L HCl. Urine urea nitrogen concentration was determined according to Bhandari et al. [8]. Urine creatinine (picric acid method) and uric acid (uricase method) concentrations were determined using kits purchased from Nanjing Jiancheng Bioengineering Institute. Urine allantoin concentration was determined using a colorimetric method [11]. Rumen microbial protein content was determined using the purine derivatives (PD) method [12]. The calculation formulas were:

$$\text{Urine volume} = 29 \times \text{body weight/creatinine [13]}$$

$$\text{Allantoin (uric acid) excretion} = \text{allantoin (uric acid) concentration} \times \text{urine volume}$$

$$\text{PD excretion (mmol/d)} = \text{allantoin excretion} + \text{uric acid excretion}$$

$$\text{PD absorption (mmol/d)} = (\text{PD excretion} - 0.385 \times \text{BW}^{0.75})/0.85$$

$$\text{Microbial protein yield (mmol/d)} = (\text{PD absorption} \times 70)/(0.116 \times 0.83 \times 1000)$$

where $0.385 \times \text{BW}^{0.75}$ is endogenous PD excretion (mmol/d), 0.85 is PD absorption rate, 70 is nitrogen content in PD (mg/mmol), 0.116 is the ratio of PD nitrogen to total rumen microbial nitrogen, and 0.83 is the digestibility of microbial purines.

Blood Collection and Analysis On day 56 of the trial, tail vein blood was collected before morning feeding after overnight fasting. EDTA-anticoagulated blood was prepared and centrifuged at 3,000 r/min for 20 min to obtain plasma. Blood urea nitrogen concentration was determined according to Bhandari et al. [8].

Data Processing and Statistical Analysis Experimental data were analyzed using SAS 9.2 statistical software. Respiratory rate, rectal temperature, milk yield, energy-corrected milk, 4% fat-corrected milk, feed-to-milk ratio, and milk composition were analyzed using the Proc Mixed model, while other indicators were analyzed using t-tests in the General Linear Model (GLM). $P<0.05$ was considered statistically significant.

Results

Barn THI and Changes in Respiratory Rate and Rectal Temperature of Dairy Cows The THI in the barn during the entire trial period is shown in Figure 1 [Figure 1: see original paper]. The average THI values at 06:00, 14:00, and 22:00 were 73.8 (65.7-82.0), 80.0 (67.9-86.9), and 77.0 (65.8-86.6), respectively, indicating that cows were under heat stress (THI > 72) during the trial period. As shown in Table 2 , there were no significant differences in respiratory rate or rectal temperature between the two groups ($P>0.05$).

Effects of Forage Combinations on Production Performance of Heat-Stressed Dairy Cows As shown in Table 3 , the AF group had highly significantly higher milk yield, milk protein yield, and lactose yield than the CS group ($P<0.01$). Energy-corrected milk and 4% fat-corrected milk were significantly higher in the AF group ($P<0.05$). However, there were no significant differences in feed-to-milk ratio or milk fat yield ($P>0.05$).

Effects of Forage Combinations on Nutrient Intake and Apparent Digestibility in Heat-Stressed Dairy Cows As shown in Table 4 , compared with the CS group, the AF group had significantly higher apparent digestibility of dry matter and organic matter ($P<0.05$), and highly significantly higher intake of dry matter, organic matter, CP, and apparent digestibility of CP ($P<0.01$). However, ADF intake and apparent digestibility of NDF and ADF were significantly lower in the AF group ($P<0.01$). There were no significant differences in NDF intake, EE intake, or EE apparent digestibility between groups ($P>0.05$).

Effects of Forage Combinations on Blood, Urine, and Milk Urea Nitrogen Concentrations and Rumen Microbial Protein Yield in Heat-Stressed Dairy Cows As shown in Table 5 , blood and urine urea nitrogen concentrations in the AF group were highly significantly lower than in the CS group ($P<0.01$), while rumen microbial protein yield was significantly higher ($P<0.05$). There was no significant difference in milk urea nitrogen concentration between groups ($P>0.05$).

Effects of Forage Combinations on Nitrogen Conversion Efficiency in Heat-Stressed Dairy Cows As shown in Table 6 , nitrogen intake and milk nitrogen yield in the AF group were highly significantly higher than in the CS group ($P<0.01$), but nitrogen conversion efficiency was significantly lower ($P<0.05$).

Discussion

Heat Stress Indicators THI is considered an important indicator for assessing heat stress in dairy cows. Studies have shown that when THI exceeds 72, cows experience severe stress responses [14]. During the trial period, THI was mostly above 72, with THI at 14:00 even exceeding 78, indicating that cows were under heat stress. Additionally, Berman et al. [15] reported that when respiratory rate exceeds 60 breaths/min, heat-stressed cows exhibit severe negative reactions. The respiratory rate at 14:00 in this trial exceeded 60 breaths/min, further confirming that cows were experiencing heat stress.

Effects of Forage Combinations on Production Performance of Heat-Stressed Dairy Cows Heat stress reduces feed intake in dairy cows, leading to decreased nutrient intake and lower milk yield [16]. Baumgard et al. [17]

reported that under heat stress, cows prioritize nutrient utilization for tissue maintenance rather than milk production. In this trial, compared with the CS group, the AF group altered forage combinations, improved diet palatability, and increased feed intake. Ren et al. [6] also found that cows fed alfalfa hay + corn silage diets had higher feed intake than those fed corn straw diets. Due to increased feed intake, milk yield in the AF group increased accordingly. Wang et al. [18] found that milk yield in cows fed alfalfa hay diets was significantly higher than in those fed corn straw diets. Additionally, the higher CP content in the AF group diet may be another reason for the increased milk yield. Grings et al. [19] found that milk yield increased when dietary CP level was elevated.

Effects of Forage Combinations on Nutrient Apparent Digestibility in Heat-Stressed Dairy Cows Heat stress can reduce nutrient digestibility in dairy cows [20]. Wen et al. [21] found that heat stress decreased CP digestibility by 10% in dairy cows. This trial investigated the effects of different forage combinations on CP apparent digestibility under heat stress conditions. The results showed that CP digestibility in the AF group diet was higher than in the CS group diet, indicating that high CP intake can improve CP digestibility. This result is consistent with Zhou et al. [22], who found that apparent digestibility of CP and nitrogen increased with dietary CP level. Chen et al. [23] suggested that higher CP content in forage is beneficial for CP degradation, while the cellulose structure of straw affects protein breakdown.

Effects of Forage Combinations on Rumen Microbial Protein Synthesis in Heat-Stressed Dairy Cows Rumen microbial protein is an important component of metabolizable protein and affects the efficiency of converting dietary CP to milk protein [24]. Dietary protein is degraded into ammonia nitrogen in the rumen for microbial utilization [25]. However, excess ammonia nitrogen passes through the rumen wall into the blood, is converted to urea in the liver, and is excreted in urine. This not only affects rumen microbial growth and wastes nitrogen resources but also pollutes the environment [5]. This trial found that compared with the CS group, the AF group had higher rumen microbial protein yield and lower blood and urine urea nitrogen concentrations. Therefore, compared with corn straw diets, alfalfa hay + corn silage diets are more suitable for rumen microbial growth in heat-stressed dairy cows.

Effects of Forage Combinations on Nitrogen Conversion Efficiency in Heat-Stressed Dairy Cows Milk protein yield is closely related to protein intake and microbial protein yield. Wright et al. [26] found that as dietary CP level increased, sufficient nitrogen was released to synthesize rumen microbial protein, thereby increasing milk protein yield. Zhao et al. [24] reported that the correlation coefficient between milk protein yield and CP intake was 0.874, and that with microbial protein yield was 0.484. In this trial, the AF group had higher nitrogen intake and milk nitrogen yield but lower nitrogen conversion efficiency. The low nitrogen conversion efficiency may be due to the high CP level (18.5%), which increased urinary and fecal nitrogen excretion. Mulligan et al. [27] found that cows fed high-protein diets (20.4%) had higher urinary and total nitrogen excretion than those fed low-protein diets (16.4%). The results of this trial indicate that compared with corn straw, the combination of alfalfa hay and corn silage increased dietary CP level, increased CP intake and digestibility, promoted microbial protein and milk protein synthesis, and increased milk yield. However, the high CP level was not conducive to efficient nitrogen conversion.

Conclusion

During summer, dairy cows experience heat stress, which significantly reduces feed intake, leading to decreased nutrient intake and milk yield. Additionally, heat stress alters nitrogen metabolism, increasing protein requirements for maintenance and reducing milk protein synthesis. This trial demonstrated that feeding alfalfa hay + corn silage diets increased feed intake and CP apparent digestibility in heat-stressed dairy cows, promoted rumen microbial protein and milk protein synthesis, and increased milk yield. However, the nitrogen conversion efficiency of the alfalfa hay + corn silage diet was relatively low, and further research is needed to determine the optimal CP level to promote milk protein synthesis and improve nitrogen conversion efficiency in heat-stressed dairy cows.

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