Effects of Different Dietary Patterns on Mammary Uptake of Long-Chain Fatty Acids in Dairy Cows: Postprint

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Abstract

This experiment aimed to investigate the effects of different dietary patterns on the uptake of long-chain fatty acids by the mammary gland in dairy cows. Thirty healthy Holstein dairy cows with similar parity, body weight [(554±21) kg], lactation period [(120±4) d], and milk yield [(24.30±1.47) kg/d] were selected and randomly assigned to three groups (n=10 per group) using a randomized block design. The three groups were fed different dietary patterns: a diet with alfalfa, Chinese wild rye, and whole-plant corn silage as forage (MF group), a diet with similar nutrient levels to the MF group but using corn stover as the sole forage (CS1 group), and a diet with the same forage proportion as the MF group but using corn stover as the sole forage (CS2 group). The experimental period lasted 90 d, divided into three periods of 30 d each. During the last 2 d of each period, feed, milk, and blood samples were collected for analysis. The results showed that: 1) Different dietary patterns had no significant effects on body weight and dry matter intake ($P > 0.05$), but significantly affected milk yield, milk fat percentage, and daily milk fat yield ($P < 0.05$). Milk yield and daily milk fat yield in the MF group were significantly higher than those in the CS1 and CS2 groups ($P < 0.05$), while the CS1 group was significantly higher than the CS2 group ($P < 0.05$). The milk fat percentage in the MF group was significantly higher than that in the CS1 group ($P < 0.05$). 2) Different dietary patterns had no significant effects on the concentrations of C16:0, C18:0, C18:2cis-6, C18:1cis-9, and total long-chain fatty acids in arterial and venous plasma ($P > 0.05$). 3) Different dietary patterns significantly affected blood flow ($P < 0.05$), showing a trend of CS1 group > MF group > CS2 group. Different dietary patterns affected the arterial supply of total long-chain fatty acids, with the MF and CS1 groups being significantly greater than the CS2 group (5,629.51 and 6,605.02 g/d vs. 3,878.91 g/d) ($P < 0.05$). 4) Different dietary patterns affected the uptake rate of total long-chain fatty acids by the mammary gland, with the MF and CS2 groups being significantly higher than the CS1 group (10.99% and

10.84% vs. 7.39%) ($P < 0.05$). 5) Different dietary patterns affected the uptake amount of total long-chain fatty acids by the mammary gland, showing MF group (618.69 g/d) > CS1 group (487.87 g/d) > CS2 group (420.56 g/d), with significant differences among groups ($P < 0.05$). This study revealed that under low-quality forage conditions, increasing concentrate level could not effectively enhance the uptake of long-chain fatty acids in dairy cows.

Full Text

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Abstract: This experiment investigated the effects of different dietary patterns on mammary uptake of long-chain fatty acids in dairy cows. Thirty healthy Holstein cows with similar parity, body weight [(554±21) kg], lactation stage [(120±4) d], and milk yield [(24.30±1.47) kg/d] were randomly assigned to three groups (n=10) using a randomized block design. The three groups received different dietary patterns: a diet with alfalfa, Leymus chinensis, and whole corn silage as roughage sources (MF group); a diet with corn straw as the sole roughage source but similar nutrient levels to the MF group (CS1 group); and a diet with corn straw as the sole roughage source but the same roughage-to-concentrate ratio as the MF group (CS2 group). The 90-day experiment consisted of three 30-day periods, with diet, milk, and blood samples collected during the final two days of each period for analysis. The results showed that: (1) Dietary patterns had no significant effects on body weight or dry matter intake ($P>0.05$), but significantly affected milk yield, milk fat percentage, and daily milk fat yield ($P<0.05$). The MF group exhibited significantly higher milk yield and daily milk fat yield compared to both CS1 and CS2 groups ($P<0.05$), while CS1 was significantly higher than CS2 ($P<0.05$). Milk fat percentage in the MF group was significantly higher than in CS1 ($P<0.05$). (2) Dietary patterns did not significantly affect plasma concentrations of C16:0, C18:0, C18:2cis-6, C18:1cis-9, or total long-chain fatty acids in arterial or venous blood ($P>0.05$). (3) Dietary patterns significantly influenced mammary blood flow ($P<0.05$), following the pattern CS1 > MF > CS2. Arterial supply of total long-chain fatty acids was significantly greater in MF and CS1 groups compared to CS2 (5,629.51 and 6,605.02 g/d vs. 3,878.91 g/d) ($P<0.05$). (4) Mammary extraction rate of total long-chain fatty acids was significantly higher in MF and CS2 groups than in CS1 (10.99% and 10.84% vs. 7.39%) ($P<0.05$). (5) Mammary uptake of total long-chain fatty acids followed the pattern MF group (618.69 g/d) > CS1 group (487.87 g/d) > CS2 group (420.56 g/d), with significant differences among groups ($P<0.05$). This study reveals that under low-quality roughage conditions, simply increasing concentrate proportion is not

an effective strategy to enhance mammary uptake of long-chain fatty acids.

Keywords: diet; dairy cows; long-chain fatty acids; uptake

Introduction

China' s dairy industry has achieved remarkable progress, yet dairy farming has primarily developed through quantitative expansion, resulting in insufficient supply of high-quality roughage. In most regions, crop straw remains the main roughage source for dairy cows, leading to different dietary patterns characterized by unreasonable dietary structure and nutrient levels, which consequently produce generally low milk fat content. Elgersma et al. [**?**] demonstrated that milk fat is primarily synthesized from long-chain fatty acids (>C16) and medium-chain (C10–C14) and short-chain (C4–C8) fatty acids, with most long-chain fatty acids being directly absorbed from the diet and reflected in milk composition. While research on milk fatty acid composition has focused extensively on rumen fermentation and small intestine absorption with substantial supporting theory, studies on mammary gland uptake remain scarce [**?**]. Current dietary patterns in China' s dairy industry mainly include three models: (1) large-scale dairy farm model using high-quality mixed roughage with relatively low concentrate supplementation; (2) dairy community model using corn straw with high concentrate supplementation; and (3) smallholder model using corn straw with minimal concentrate supplementation. This experiment aimed to investigate the effects of these different dietary patterns on mammary uptake of long-chain fatty acids to provide a theoretical basis for effective milk fat synthesis.

Materials and Methods

Experimental Animals and Management

Thirty healthy Holstein cows (2–3 parity) with similar body weight [(554±21) kg], lactation stage [(120±24) d], and milk yield [(24±1.47) kg/d] were used. Cows had ad libitum access to feed, with approximately 5% refusals maintained daily. Total mixed rations (TMR) were fed twice daily at 06:00 and 18:00, with free access to water. Milking occurred once daily before feeding (06:00 and 18:00). Feed intake and milk yield were recorded daily throughout the experiment.

Experimental Design and Diets

A randomized block design was employed with three groups (n=10). The MF group received a diet with a 45:55 concentrate-to-roughage ratio containing mixed high-quality roughage (alfalfa + whole corn silage + Leymus chinensis). The CS1 group received a diet with corn straw as the sole roughage source, a

65:35 concentrate-to-roughage ratio, and nutrient levels approximately equivalent to the MF group. The CS2 group received a diet with corn straw as the sole roughage source but maintaining the same 45:55 concentrate-to-roughage ratio as the MF group. All groups received the same concentrate formulation. Diet composition and nutrient levels are presented in Table 1 . The 90-day experiment comprised three 30-day periods, with diet, milk, and plasma samples collected during the final two days of each period for analysis. The MF group served as the control to evaluate the effects of long-term feeding of CS1 and CS2 diets on mammary uptake of long-chain fatty acids.

Sample Collection and Analysis

Diet Sampling and Analysis During the final two days of each period, diet samples were collected in the morning and evening, dried at 65°C to determine initial moisture content, and calculate dry matter intake (DMI). After the two-day collection, samples were thoroughly mixed, quartered, and approximately 500 g was ground through a 40-mesh sieve for routine nutrient analysis, including crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and starch content, with net energy for lactation (NEL) calculated. Crude protein, ether extract, NDF, and ADF were determined according to AOAC (1999) [**?**] using a FOSS Kjeltec Auto Analyzer, SZC-101 Soxhlet extractor, and ANKOM fiber analyzer, respectively. Starch and energy were analyzed using a FOSS NIRS DS 2500 near-infrared spectroscopy analyzer at Huaxia Dairy (Sanhe) Co., Ltd. Starch content was measured directly, while NEL was estimated by summing values for individual dietary ingredients according to their formulation proportions.

Milk Sampling and Analysis Milk samples were collected during the final two days of each period from morning and evening milkings, mixed proportionally based on the average milk yield ratio from the three days preceding sampling, and stored at -20°C. Milk fat percentage was determined using a MilkoScanTM Minor Type 78110 (FOSS Analytical A/S 69, DK-3400, Denmark).

Blood Sampling and Analysis Blood samples were collected during the final two days of each period. On day 1, 20 mL of arterial and venous blood was collected from the tail vessels using heparinized vacuum tubes before morning and afternoon feeding (0 h). On day 2, the same procedure was performed at 6 h after morning and afternoon feeding. Samples were centrifuged at 4,000×g for 15 min at 4°C to separate plasma, which was aliquoted into 1.5 mL tubes and stored at -20°C. After the experiment, plasma samples from the four time points were pooled equally into 10 mL tubes and stored at -20°C until analysis.

Fatty Acid Composition Analysis of Diet, Milk, and Plasma Samples Lipids were extracted using a mixture of hexane and isopropanol, followed by

acid-base methylation of the hexane lipid fraction. Sample processing and external standard analysis methods followed Khas-Erdene et al. [**?**]. Fatty acid methyl esters (FAME) were quantified by gas chromatography (GC-2014, Shimadzu, Japan) equipped with a flame ionization detector. Diluted samples (50:1) were injected using a 2 L microsyringe into a HP-88 capillary column (100 m × 0.25 mm, 0.20 m film thickness, Agilent Technologies). The oven temperature was initially held at 120°C for 10 min, then increased at 3.2°C/min to 230°C and held for 35 min. Injector and detector temperatures were maintained at 250°C and 300°C, respectively. Total analysis time was 79.38 min per sample. Qualitative external standard calibration was performed using 37-component FAME standards (Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA; Supelco 37 Component FAME Mix, Supelco Inc., USA).

Fatty acid concentrations were calculated using peak areas [**?**]: - Total FAME concentration (mg/mL) = [(Total peak area - Internal standard peak area) × Known internal standard concentration] / Internal standard peak area - Fi = (External standard i concentration × Known internal standard peak area × Known internal standard concentration) / External standard i peak area - Individual FAME concentration (mg/mL) = (Ai × Fi × Known internal standard concentration) / Known internal standard peak area

Where external standard i represents the ith fatty acid external standard, Ai is the peak area of the ith fatty acid, and Fi is the relative correction factor for the ith fatty acid.

Fatty Acid Uptake Calculations [?]

- Mammary blood flow (MBF, L/h) = Milk concentration (C18:0 + C18:1cis-9) / [Arterial plasma concentration (C18:0 + C18:1cis-9) - Venous plasma concentration (C18:0 + C18:1cis-9)] × Milk yield / 24 h
- Arterial supply (g/d) = Arterial plasma concentration × Blood flow × 24 h
- Mammary extraction rate (%) = [(Arterial plasma concentration - Venous plasma concentration) / Arterial plasma concentration] × 100
- Mammary uptake (g/d) = (Arterial plasma concentration - Venous plasma concentration) × Blood flow × 24 h

Statistical Analysis

Data were analyzed as repeated measures using the PROC MIXED procedure of SAS 9.0 software. Treatment effects were considered fixed, while cows were treated as random effects. Dry matter intake was used as a covariate throughout the experiment. Data are presented as covariate-adjusted least squares means with pooled standard error of the mean (SEM). Significance was declared at $P<0.05$.

Results

Effects of Different Dietary Patterns on DMI, Milk Yield, and Milk Fat

After 90 days of feeding the three experimental diets to mid-lactation cows, results (Table 2) showed that dietary patterns had no significant effects on body weight or DMI ($P>0.05$), but significantly affected milk yield, milk fat percentage, and daily milk fat yield ($P<0.05$). The MF group exhibited significantly higher milk yield and daily milk fat yield compared to both CS1 and CS2 groups ($P<0.05$), while CS1 was significantly higher than CS2 ($P<0.05$). Milk fat percentage in the MF group was significantly higher than in CS1 ($P<0.05$), with CS2 intermediate and not significantly different from either group ($P>0.05$). These results indicate that diets with corn straw as the sole roughage source reduced milk yield and milk fat production. Although increasing concentrate proportion in corn straw-based diets improved milk yield and milk fat production, performance remained inferior to diets with high-quality roughage at similar nutrient levels.

Effects of Different Dietary Patterns on Long-Chain Fatty Acid Concentrations in Tail Artery and Vein Plasma

The main fatty acid concentrations in the experimental diets are shown in Table 3 . As shown in Table 4 , dietary patterns had no significant effects on plasma concentrations of C16:0, C18:0, C18:2cis-6, C18:1cis-9, or total long-chain fatty acids in arterial or venous blood ($P>0.05$). However, C18:3n3 concentrations in arterial and venous plasma were significantly higher in CS1 and CS2 groups compared to MF ($P<0.05$), with no significant difference between CS1 and CS2 ($P>0.05$). The relative concentrations of individual long-chain fatty acids in plasma (Table 4) generally reflected their corresponding concentrations in the diets (Table 3).

Effects of Different Dietary Patterns on Mammary Blood Flow, Arterial Supply, and Extraction Rate of Long-Chain Fatty Acids

As shown in Table 5 , dietary patterns significantly affected mammary blood flow ($P<0.05$), following the pattern CS1 > MF > CS2. Although differences in plasma long-chain fatty acid concentrations between arterial and venous blood were relatively small (Table 4), arterial supply of all long-chain fatty acids showed strong consistency with blood flow patterns, also following CS1 > MF > CS2. Overall, mammary extraction rates of long-chain fatty acids were higher in CS2 and MF groups compared to CS1. Specifically, extraction rates for C18:1cis-9 showed an increasing trend ($P<0.10$), while extraction rates for C16:0, C18:3n3, and total long-chain fatty acids were significantly higher than CS1 ($P<0.05$), with no significant differences between CS2 and MF ($P>0.05$). These results indicate that while the CS1 diet increased fatty acid supply to the mammary gland, it significantly reduced mammary extraction rates. Con-

versely, despite higher extraction rates in CS2 cows, dietary supply of long-chain fatty acids was insufficient.

Effects of Different Dietary Patterns on Mammary Uptake of Long-Chain Fatty Acids

As shown in Table 6 , mammary uptake of C16:0, C18:2cis-6, and total long-chain fatty acids followed the pattern MF > CS1 > CS2, with significant differences among groups ($P<0.05$). Uptake of C18:0 in CS2 was significantly lower than in the other two groups ($P<0.05$), which did not differ significantly from each other ($P>0.05$). Uptake of C18:1cis-9 and C18:3n3 was significantly higher in CS1 and CS2 compared to MF ($P<0.05$), with no significant difference between CS1 and CS2 ($P>0.05$). These results demonstrate that MF cows had greater mammary uptake of long-chain fatty acids compared to CS1 and CS2, with CS1 intermediate.

Discussion

Effects of Different Dietary Patterns on DMI, Milk Yield, and Milk Fat

Dry matter intake is a critical determinant of dairy cow performance. Llamas-Lamas and Combs [**?**] reported that feeding diets with 86%, 71%, and 56% concentrate with silage resulted in highest DMI for the 86% concentrate group, with no significant difference between the 71% and 56% groups. The current experiment employed similar concentrate-to-roughage ratios and feeding conditions, with no significant differences in DMI observed among the three dietary patterns.

Dietary DMI, concentrate-to-roughage ratio, and roughage source can all influence milk yield. Krause et al. [**?**] demonstrated that low-quality roughage diets reduce feed digestibility and nutrient uptake capacity, thereby decreasing milk yield and milk fat production, though increasing dietary concentrate proportion can improve these parameters. In the present study, milk yield and daily milk fat yield differed significantly among groups, following the pattern MF > CS1 > CS2, consistent with Krause et al. [**?**].

These results suggest that cows in this experiment may have reached their maximum DMI capacity, explaining the lack of significant differences. Furthermore, when consuming low-energy, low-nitrogen diets with corn straw as the sole roughage source (smallholder model), cows did not show significant body weight loss but instead markedly reduced milk yield to decrease lactation demands.

Milk fat is an important nutritional indicator. Research indicates that high-grain diets can cause milk fat depression, characterized by low milk fat percentage and yield [**?**], while increasing dietary neutral detergent fiber levels significantly elevates milk fat percentage [**?**], consistent with our findings. Low-fiber,

high-grain diets can induce milk fat depression syndrome (<3.7 g/L) [?]. The CS1 diet, despite its high concentrate content, maintained adequate fiber levels, likely preventing milk fat depression syndrome, though the milk fat percentage of 3.71% approached the critical threshold, indicating high risk.

Effects of Different Dietary Patterns on Mammary Uptake of Long-Chain Fatty Acids

Blood fatty acid concentrations significantly influence mammary fatty acid metabolism. Research shows that changes in blood fatty acid concentrations largely originate from alterations in dietary nutrient composition, with blood fatty acid concentrations responding linearly to corresponding dietary fatty acid levels. Despite rumen biohydrogenation, increasing specific dietary fatty acids linearly increases their concentration and proportion in blood and milk [?]. Our results align with these findings, particularly for linolenic acid (C18:3n3), which showed significant dietary effects.

Mammary blood flow is a critical controlling factor for fatty acid uptake. Studies indicate that while DMI does not significantly affect blood flow, increasing dietary starch and protein supply or infusing amino acids into blood significantly enhances blood flow [?]. In this study, blood flow followed the pattern CS1 > MF > CS2, consistent with the higher starch and protein levels in the CS1 diet. Arterial fatty acid supply is influenced by both plasma fatty acid concentration and blood flow [?]. Since dietary patterns did not significantly affect plasma long-chain fatty acid concentrations but significantly influenced blood flow, arterial supply patterns mirrored blood flow patterns.

Mammary uptake of fatty acids is determined by both extraction rate and arterial supply, with extraction rate being influenced by blood flow; increased blood flow often accompanies decreased nutrient extraction rates [?]. Zhang et al. [?] reported that increasing dietary and blood fatty acid supply reduces extraction efficiency and transport rates while increasing their concentration and yield in milk. Our results showed that CS1 cows, despite having the highest blood flow and fatty acid supply, exhibited significantly reduced mammary extraction rates, yet their uptake of long-chain fatty acids was significantly greater than CS2, consistent with previous research. Additionally, studies show that high-quality roughage improves the efficiency of fatty acid transfer into milk, thereby increasing milk fat content and yield while maintaining higher milk fat percentage [?]. In our study, MF cows showed improved extraction rates for long-chain fatty acids compared to CS1, consequently increasing mammary uptake. The discrepancy in C16:0 extraction rate may be explained by the fact that approximately half of C16:0 originates from de novo synthesis in the mammary gland.

In conclusion, compared to high-quality roughage diets, the CS1 diet (increased concentrate proportion) reduced mammary extraction rates and consequently decreased mammary uptake of long-chain fatty acids. The CS2 diet (reduced

nutrient level) decreased arterial supply of long-chain fatty acids, also reducing mammary uptake. These phenomena may similarly affect relationships between milk component precursors and milk yield and composition, warranting further investigation. The reduced mammary extraction rate of long-chain fatty acids is likely associated with decreased milk fat percentage.

Different dietary patterns affected milk yield, milk fat production, milk fat percentage, and mammary uptake of long-chain fatty acids. Simply increasing concentrate proportion in low-quality roughage diets reduces mammary extraction efficiency and is not an effective approach to enhance long-chain fatty acid uptake in dairy cows.

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