Effects of Different Diets on Uptake and Utilization of Milk Fat Precursors in Lactating Dairy Cows: Postprint

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Abstract

This experiment aimed to investigate the uptake and utilization patterns of milk fat precursors in lactating dairy cows under different dietary conditions. Nine healthy lactating Holstein dairy cows with a body weight of (617±21) kg and days in milk of (120±20) d were selected as experimental animals, and a 3 replicated 3×3 Latin square design was adopted to randomly assign them into 3 groups. Two groups had different concentrate formulas but the same roughage (straw) with a concentrate to roughage ratio of 6:4 (designated as CSA and CSB groups), while the remaining group received mixed roughage consisting of Leymus chinensis, alfalfa hay, and whole-plant corn silage with a concentrate to roughage ratio of 4:6 (MF group), with 3 cows per group; the experimental period lasted 84 d, divided into 3 periods of 28 d each, including a 21 d preliminary period and a 7 d formal experimental period. The results showed that: 1) Milk yield and milk composition were not significantly different among groups (P>0.05), but lactation efficiency in the MF group was significantly higher than that in the CSA and CSB groups (P<0.05). 2) Dietary intake of C16:0 and C18:0 was not significantly different among groups (P>0.05); dietary intake of C18:1 c9 in the MF group was significantly lower than that in CSA and CSB groups (P<0.05). 3) The contents of long-chain fatty acids and total fatty acids in arterial plasma were higher in the MF group than in CSA and CSB groups, especially C18:3 n3 content was significantly higher (P<0.05). 4) Mammary uptake of C18:3 n3 (daily average) in MF and CSB groups was significantly higher than that in the CSA group (P<0.05); mammary uptake of other long-chain fatty acids was not significantly different among groups (P>0.05). 5) This was ultimately reflected in milk, showing that C18:3 n3 yield in the MF group tended to be higher compared with CSA and CSB groups (0.05 P<0.10), whereas C18:2 n6, which had no significant difference in mammary uptake (P>0.05), was significantly lower in milk yield than CSA and CSB groups (P<0.05). It can be concluded that diets with the same roughage but different concentrates had no

significant effect on production performance of lactating dairy cows, whereas quality roughage could still affect the composition and yield of fatty acids in milk even when the proportion of concentrate was reduced, making it more beneficial to human health.

Full Text

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Abstract

This study investigated the uptake and utilization patterns of milk fat precursors in lactating dairy cows under different dietary conditions. Nine healthy Holstein cows with body weight of (617±21) kg and lactation days of (120±20) d were selected as experimental animals. Using a 3-replicate 3×3 Latin square design, the cows were randomly divided into three groups: two groups received different concentrate formulations but the same corn straw roughage at a concentrate-to-roughage ratio of 6:4 (designated CSA and CSB groups), while the remaining group received a mixed roughage of Chinese wildrye, alfalfa hay, and whole-plant corn silage at a concentrate-to-roughage ratio of 4:6 (MF group), with three cows per group. The 84-day experiment consisted of three periods, each lasting 28 days (21 days of pre-trial and 7 days of formal trial). The results showed that: (1) No significant differences were observed in milk yield or milk composition among groups ($P>0.05$), but the dairy efficiency of the MF group was significantly higher than that of the CSA and CSB groups ($P<0.05$). (2) Dietary intake of C16:0 and C18:0 showed no significant differences among groups ($P>0.05$), while dietary C18:1 c9 intake in the MF group was significantly lower than in the CSA and CSB groups ($P<0.05$). (3) The MF group exhibited higher contents of long-chain fatty acids and total fatty acids in arterial plasma compared to the CSA and CSB groups, particularly with significantly higher C18:3 n3 content ($P<0.05$). (4) Mammary uptake of C18:3 n3 (daily average) in the MF and CSB groups was significantly higher than in the CSA group ($P<0.05$), while mammary uptake of other long-chain fatty acids showed no significant differences among groups ($P>0.05$). (5) Ultimately reflected in milk, C18:3 n3 yield in the MF group tended to be higher than in the CSA and CSB groups (0.05 $P<0.10$), while C18:2 n6 yield was significantly lower ($P<0.05$) despite no significant difference in mammary uptake ($P>0.05$). These findings indicate that while different concentrate formulations with the same roughage source did not significantly affect lactation performance, high-quality roughage combinations could still influence milk fatty acid composition and yield even with reduced concentrate proportions, shifting the profile toward greater benefits for

human health.

Keywords: lactating dairy cow; roughage; milk fat; fatty acid; mammary gland uptake

1. Materials and Methods

1.1 Experimental Design and Animal Management

Nine healthy multiparous Holstein cows in their 2nd–3rd lactation, with body weight of (617±21) kg and lactation days of (120±20) d, were selected for this experiment. A 3-replicate 3×3 Latin square design was employed, with nine cows randomly divided into three groups. Two groups received different concentrate formulations but identical corn straw roughage at a concentrate-to-roughage ratio of 6:4 (designated CSA and CSB groups), while the remaining group received a mixed roughage of Chinese wildrye, alfalfa hay, and corn silage at a concentrate-to-roughage ratio of 4:6 (MF group), with three cows per group. The experimental diets met NRC (2001) nutritional standards, with composition and nutrient levels shown in Table 1 . The 84-day experiment consisted of three periods, each lasting 28 days (including 21 days of pre-trial and 7 days of formal trial).

The experiment was conducted at the Bingzhouhai Dairy Cooperative Demonstration Ranch of Inner Mongolia Dairy Union Technology Co., Ltd. Each cow was individually housed with an independent exercise yard, fed in TMR form, with individual records of feed intake and refusals. Milking was performed using a Lely portable mobile milking machine. The milking machine, cow beds, and exercise yards were regularly cleaned and disinfected. Cows were fed twice daily (07:00 and 19:00) with ad libitum water access and milked twice daily (06:00 and 18:00).

1.2 Sample Collection

Daily milk yield was accurately recorded throughout the trial. Milk samples were collected on days 1–3 of each formal trial period, with approximately 300 g of milk sample collected from each cow after milking. One portion was used for milk composition determination, while the remainder was stored at -20 °C for milk fatty acid content analysis.

Blood sample collection was conducted within two days after milk sample collection (days 4–5 of the formal trial period). On the first sampling day, fasting blood was collected twice from each cow—before morning feeding and before afternoon feeding—using anticoagulant tubes to collect blood from the caudal artery and mammary vein for plasma preparation, which was stored at -20 °C. On the second sampling day, caudal artery and mammary vein blood were collected 2–3 h after morning feeding, centrifuged at 1,500 ×g for 10 min, and plasma samples were prepared and stored at -20 °C.

1.3 Calculations and Laboratory Analyses

1.3.1 Calculation of Mammary Blood Flow, Precursor Uptake Rate, and Mammary Uptake Using C18:0 + C18:1 c9 as an endogenous marker to estimate mammary blood flow, following the method of Annison et al. [6]:

Blood flow = Milk content of C18:0 and C18:1 c9 / (Arterial plasma content of C18:0 and C18:1 c9 –Venous plasma content of C18:0 and C18:1 c9)

The extraction rate, mammary uptake, and mammary uptake balance of milk component precursors were calculated according to Enjalbert et al. [7]:

Extraction rate (%) = [(Arterial plasma content of a precursor –Venous plasma content of the precursor) / (Arterial plasma content of the precursor)] × 100

Mammary uptake (mmol/L) = (Arterial plasma content of a precursor –Venous plasma content of the precursor) × Blood flow

Mammary uptake balance (mmol/L) = Milk content of a precursor –Mammary uptake

1.3.2 Milk Composition Determination Milk protein, milk fat, lactose, and total solids contents were determined using a MilkoScanTM minor-Foss milk composition analyzer.

1.3.3 Determination of Fatty Acid Content in Plasma and Milk
1.3.3.1 Plasma Sample Preparation

The procedure was as follows: (1) 1 mL of plasma was placed in a 10 mL centrifuge tube, and 5 mL of n-hexane-isopropanol mixture (V/V = 3/2) was added and vortexed for 2 min. (2) The upper organic phase (as completely as possible) was transferred to a hydrolysis tube and dried under nitrogen gas, then 0.5 mL of n-hexane and 1 mL of anhydrous methanol were added. (3) 3 mL of 2% sodium hydroxide methanol solution (2 g NaOH dissolved in 100 mL anhydrous methanol) was added, and the mixture was saponified in a 50 °C water bath for 30 min. (4) After cooling to room temperature, 3 mL of 10% hydrochloric acid methanol solution was added, the hydrolysis tube was tightly capped, and the mixture was esterified in a 90 °C water bath for 2 h. (5) After cooling to room temperature, 3 mL of water and 5 mL of n-hexane were added, shaken, and allowed to stand for layer separation. (6) The upper liquid (as completely as possible) was taken, dried under nitrogen gas to near dryness, diluted to 1 mL with n-hexane, vortexed for 30 s, and approximately 0.5 g of anhydrous sodium sulfate was added to absorb water before sample analysis. (Note: All liquid reagents used were chromatographic grade, and water used was ultrapure. Liquid transfer was performed as completely as possible to ensure accurate results.)

1.3.3.2 Milk Sample Preparation

The procedure was as follows: (1) 2 mL of milk sample was mixed with 4 mL of n-hexane-isopropanol mixture (V/V = 3/2), sodium sulfate solution (6.67 g anhydrous sodium sulfate dissolved in 100 mL water) was added, and the mixture was centrifuged at 2,000 ×g for 20 min at room temperature. (2) The upper organic phase was transferred to a 20 mL capped hydrolysis tube and dried under nitrogen gas. (3) 2 mL of sodium hydroxide methanol solution (prepared as in section 1.3.3.1 for plasma pretreatment) was added, and the mixture was placed in a 50 °C water bath for 15 min, cooled, then hydrochloric acid methanol solution was added and kept in an 80 °C constant temperature water bath for 90 min. (4) After cooling to room temperature, 3 mL of water and 6 mL of n-hexane were added, shaken, and allowed to stand for layer separation. (5) The upper liquid was completely removed, diluted to 10 mL with n-hexane (Note: due to high fatty acid content in milk, dilution to 10 mL was equivalent to a 5-fold dilution), approximately 2 g of anhydrous sodium sulfate was added, and the supernatant was taken for analysis. Injection volume was 1 µL.

1.3.3.3 Chromatographic Analysis of Plasma and Milk Samples

Gas chromatography conditions were as follows: Shimadzu GC-2014 gas chromatograph equipped with an HP-88 column (100.00 m × 0.25 mm, 0.20 m pore size); column temperature was set at 120 °C for 10 min, then increased to 230 °C at a rate of 3.2 °C/min and maintained for 35 min; injector temperature was 250 °C; detector temperature was 300 °C; constant pressure of 190 kPa; split ratio of 1:50; high-purity nitrogen as carrier gas; injection volume of 1 µL. The standard used was a 37-component fatty acid methyl ester standard (Sigma).

1.3.4 Calculation of Lactation Efficiency and Standard Milk Lactation Efficiency

Fat-corrected milk yield (kg/d) = 0.4M + 15F

Normal milk lactation efficiency = Milk yield / Dry matter intake

Fat-corrected milk lactation efficiency = Fat-corrected milk yield / Dry matter intake

Where: M = milk yield (kg); F = milk fat percentage (kg).

1.4 Statistical Analysis

Experimental data were statistically analyzed using the MIXED procedure of SAS 9.0 software. Data for milk yield, milk composition, and milk fatty acid composition were analyzed according to the 3×3 Latin square design. The statistical model included random effects of experimental cows and fixed effects of experimental period and treatment. Statistical results for variables are presented as least squares means. Significance level was set at $P<0.05$, and Tukey's test was used for multiple comparisons.

2. Results

2.1 Effects of Different Diets on Dry Matter Intake and Milk Composition of Lactating Dairy Cows

As shown in Table 2 , body condition scores did not differ significantly among groups (P>0.05). Compared with the CSA and CSB groups, the MF group showed significantly lower dry matter intake (P<0.05). No significant differences were observed among the three groups in milk yield or fat-corrected milk yield (P>0.05), but the MF group exhibited significantly higher normal milk lactation efficiency and fat-corrected milk lactation efficiency compared to the CSA and CSB groups (P<0.05). Milk protein percentage, milk fat percentage, lactose percentage, total solids content, and yields of milk protein and milk fat showed no significant differences (P>0.05).

2.2 Effects of Different Diets on Mammary Blood Flow of Lactating Dairy Cows

As shown in Table 3 , no significant differences were observed among the three groups in mammary blood flow or the ratio of mammary blood flow to milk yield (P>0.05).

2.3 Effects of Different Diets on Fatty Acid Metabolism

2.3.1 Dietary Fatty Acid Composition and Intake As shown in Table 4 , no significant differences were observed among the three groups in dietary contents of C16:0 and C18:0 (P>0.05), and cows showed no significant differences in intake of these two fatty acids (P>0.05). Dietary C18:1 c9 content in the CSA and CSB groups tended to be higher than in the MF group (0.05 P<0.10). Dietary C18:1 c9 intake in the CSA and CSB groups was significantly higher than in the MF group (P<0.05). No significant differences were observed among the three groups in dietary C18:2 n6 content and intake (P>0.05). Dietary C18:3 n3 content in the MF group was significantly higher than in the CSA and CSB groups (P<0.05), and C18:3 n3 intake in the MF group was also significantly higher than in the other two groups (P<0.05).

2.3.2 Fatty Acid Contents in Arterial and Venous Plasma As shown in Table 5 , changes in fatty acid contents in arterial and venous plasma of cows were generally consistent with dietary fatty acid composition and intake. In the MF group, C18:3 n3 contents in both arterial and venous plasma were significantly higher than in the CSA and CSB groups (P<0.05).

2.3.3 Mammary Gland Extraction Rate and Uptake of Fatty Acids As shown in Table 6 , the MF group showed slightly higher extraction rates of total long-chain fatty acids compared to the CSA and CSB groups, but the difference was not significant (P>0.05). No significant differences were observed among groups in mammary extraction rates of individual long-chain fatty acids

(P>0.05). Regarding mammary uptake, C18:3 n3 uptake in the CSB and MF groups tended to be significantly higher than in the CSA group (0.05 P<0.10).

2.3.4 Milk Fatty Acid Composition and Yield As shown in Table 7 and Table 8 , the MF group exhibited lower contents and yields of most medium- and short-chain fatty acids (C6–C12) compared to the CSA and CSB groups, with significant differences in C10:0 and C12:0 contents and C12:0 yield (P<0.05). The MF group showed significantly higher C18:3 n3 content and yield in milk compared to the CSA and CSB groups (P<0.05), while C18:2 n6 and polyunsaturated fatty acid contents and yields were significantly lower (P<0.05).

2.3.5 Long-Chain Fatty Acid Flux As shown in Table 4, dietary C18:1 c9 content in the CSA and CSB groups was significantly higher than in the MF group (P<0.05), while C18:3 n3 content was significantly lower (P<0.05). Converting mammary uptake and milk fatty acid yield to daily averages (Table 9) revealed that in the mammary gland, C18:3 n3 uptake in the MF and CSB groups was significantly higher than in the CSA group (P<0.05), with no significant differences among groups in uptake of other long-chain fatty acids (P>0.05). In milk, C18:3 n3 yield in the MF group tended to be higher than in the CSA and CSB groups (0.05 P<0.10), while C18:2 n6 yield was significantly lower (P<0.05).

3. Discussion

3.1 Effects of Different Diets on Dry Matter Intake and Milk Composition of Lactating Dairy Cows

The results showed that dry matter intake was significantly lower in the MF group compared to the CSA and CSB groups. The selected cows in this experiment produced approximately 20 kg of milk daily. The lower milk yield in the MF group and the satiety effect from the high roughage proportion may have contributed to the reduced dry matter intake. Additionally, this effect may be closely related to the inclusion of whole cottonseed in the MF group. Although most studies have observed no effect on dry matter intake when feeding untreated soybeans or whole cottonseed, Coppock et al. [8] reported a negative linear relationship between increasing whole cottonseed content in diets and dry matter intake due to the extremely high energy level of whole cottonseed, which increased net energy for lactation (NEL) intake.

Although no significant differences were observed in milk yield or fat-corrected milk yield among the three groups, the MF group showed significantly higher normal milk lactation efficiency and fat-corrected milk lactation efficiency compared to the CSA and CSB groups. Other parameters including milk protein percentage, milk fat percentage, lactose percentage, total solids content, and yields of milk protein and milk fat showed no significant differences. Under conditions of no difference in dietary crude protein and net energy for lactation intake, different roughage qualities had no effect on the mammary gland' s capacity for fat and protein synthesis.

Lactation performance in dairy cows is a complex process influenced by multiple factors. Kadegowda et al. [9] and Bremmer et al. [10] suggested that adequate exogenous fat supplementation enhances the mammary gland's ability to convert exogenous fatty acids, thereby increasing milk fat percentage and reducing milk yield, which is consistent with our findings. Numerous studies have reported that milk yield and dry matter intake increase with higher dietary concentrate proportions [11–16]. Lundquist et al. [13] found that increasing the concentrate-to-roughage ratio from 40:60 to 60:40 significantly improved milk yield without substantially changing dry matter intake. Kang [17] observed no effect of dietary patterns on dry matter intake in dairy cows. Macleod et al. [14] also found that milk yield and dry matter intake increased linearly in primiparous cows when the concentrate-to-roughage ratio was increased from 25:75 to 45:55 and then to 65:35. However, in this experiment, the MF group showed significantly reduced dry matter intake, possibly due to: (1) the inclusion of whole cottonseed as a high-quality bypass fat in the MF diet, which provided higher NEL and consequently reduced dry matter intake; (2) the satiety effect from the high roughage proportion; and (3) potential feed selection behavior. In goat studies, mixed roughage diets significantly improved milk protein and fat percentages compared to corn straw diets, likely due to the inclusion of alfalfa meal and corn silage in the mixed roughage diets [18]. When dietary nutrient levels are low, high-quality roughage combinations may increase animal dry matter intake and consequently improve milk yield, milk protein percentage, and milk fat percentage, but this effect disappears when dietary nutrient levels are increased. The reduced dry matter intake observed in this experiment may be attributed to satiety from the high roughage ratio or feed selectivity. Overall, high-quality roughage combinations improve ruminant lactation efficiency.

3.2 Effects of Different Diets on Mammary Blood Flow of Lactating Dairy Cows

Measurement of mammary blood flow is fundamental for studying mammary uptake and utilization of milk precursor components [19]. Based on a hematocrit of 1/3, the ratio of mammary blood flow to milk yield in this experiment did not reach 500, which deviates from the findings of Annison et al. [20]. Long-chain fatty acids in blood primarily originate from dietary conversion, but rumen microbial hydrogenation and other factors may cause instability in blood fatty acid content and composition, contributing to this deviation. The external pudendal artery serves as the main blood supply to the mammary gland, and studying its fatty acid content and composition is crucial for understanding mammary uptake and utilization of milk fat precursors. The mammary gland, as the terminal organ for lactation, exhibits considerable independence. The results showed that different diets had no significant effect on mammary blood flow in dairy cows.

3.3 Effects of Different Diets on Mammary Uptake and Utilization of Milk Fat Precursors in Lactating Dairy Cows

For ruminants, the external pudendal artery, as the sole pathway for mammary blood supply, directly reflects dietary regulation of milk fat precursor composition in blood. In this experiment, different diets did not significantly affect fatty acid composition in the external pudendal artery plasma of dairy cows but caused significant effects on fatty acid composition and yield in the mammary gland. Shingfield et al. [21] proposed that fatty acid transport efficiency or blood flow could cause changes in fatty acid supply and mammary uptake, leading to alterations in milk fat composition. Yang et al. [22] found that mammary blood flow and blood fatty acid content affect mammary uptake of fatty acids. Within a certain range, increasing dietary or blood fatty acid content increases mammary extraction rate, but excessively high levels reduce extraction rate [23]. This study showed that dietary C18:3 n3 content and intake were significantly higher in the MF group than in the CSA and CSB groups, while dietary C18:1 c9 content was significantly higher in the CSA and CSB groups than in the MF group. Plasma analysis revealed that fatty acid composition in blood entering the mammary gland was generally consistent with dietary fatty acid composition, indicating that dietary nutrients directly affect mammary uptake and utilization of milk fat precursors. The results showed minimal differences in medium- and short-chain fatty acid contents and yields among groups. Recent research has increasingly focused on the health effects of medium- and short-chain saturated fatty acids, as several fatty acids with fewer than 10 carbons have been shown to play positive roles in gene function regulation, antiviral activity, and cancer prevention and tumor growth inhibition. Differences among groups primarily appeared in mammary uptake of long-chain and unsaturated fatty acids. As previously mentioned, the mammary gland functions as a relatively independent terminal organ for lactation and does not simply reflect dietary fatty acid composition. Under complex gene network regulation, milk fatty acid composition is significantly influenced by genes, rumen function, and hormone levels. High-quality roughage combinations may better regulate milk fat percentage and fatty acid composition. For example, studies have shown that higher dietary ratios of monounsaturated to polyunsaturated fatty acids provide better protection against atherosclerosis and cardiovascular disease than diets rich in polyunsaturated fatty acids [24–25]. The fatty acid composition in milk from the MF group supported this conclusion. With scientific advancement, milk fat research should move beyond simply pursuing increases in specific fatty acids toward optimizing milk fat composition through dietary regulation—rumen fermentation—intestinal absorption—liver conversion—blood regulation pathways to direct ruminant milk fat composition toward greater benefits for human health. Different diets had no significant effect on fatty acid composition in the external pudendal artery blood. As the sole pathway for mammary blood supply, the composition of milk fat precursors in this artery is directly regulated by diet.

In conclusion: (1) Under conditions of different concentrate-to-roughage ratios but similar nutrient levels, feeding dairy cows diets with corn straw as the sole roughage source versus high-quality mixed roughage diets resulted in no significant differences in milk protein percentage, milk fat percentage, or yields of milk protein and milk fat. (2) Different diets had no effect on milk yield or mammary blood flow in lactating dairy cows. (3) The composition of milk fat precursors flowing through the mammary gland was consistent with dietary composition, with original differences being reduced in mammary plasma. High-quality roughage combination diets promoted milk fat synthesis by increasing mammary uptake of certain milk fat precursors.

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