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

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Date: 2017-10-11T00:00:00+00:00

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 body weight of
(617$±21)kganddaysinmilkof(120±20)dwereselectedasexperimentalanimals.A3−
replicate3×3Latinsquaredesignwasadopted,andthecowswererandomlydividedinto3groups.Twogroupshaddi
to−forageratioof6 : 4(designatedasCSAandCSBgroups,respectively).Theremaininggroupwasfedamixedr
plantcornsilagewithaconcentrate−to−forageratioof4 : 6(MFgroup),with3cowspergroup.Theexperimentalp
dpreliminaryperiodanda7 − dformaltrialperiod.Theresultsshowed :
1)Milkyieldandmilkcompositiondidnotdiffersignificantlyamonggroups(P >
0.05),butlactationefficiencyintheMFgroupwassignificantlyhigherthanintheCSAandCSBgroups(P <
0.05).2)DietaryintakeofC16 : 0andC18 : 0didnotdiffersignificantlyamonggroups(P >
0.05);dietaryintakeofC18 : 1c9intheMFgroupwassignificantlylowerthanintheCSAandCSBgroups(P <
0.05).3)Thecontentsoflong−chainfattyacidsandtotalfattyacidsinarterialplasmawerehigherintheMFgroupt
3n3content,whichwassignificantlyhigher(P < 0.05).4)MammaryuptakeofC18 :
3n3(dailyaverage)intheMFandCSBgroupswassignificantlyhigherthanintheCSAgroup(P <
0.05);mammaryuptakeofotherlong−chainfattyacidsdidnotdiffersignificantlyamonggroups(P >
0.05).5)Ultimatelyreflectedinmilk,theoutputofC18 : 3n3intheMFgroupshowedanincreasingtrendcompared
whereas C18:2 n6, which had no significant difference in mammary uptake among groups (P>0.05), had significantly lower output in milk than the 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 high-quality roughage could still affect the composition and output of fatty acids in milk even when the proportion of concentrate was reduced, making it more beneficial to human health.

Full Text

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

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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)kgandlactationdaysof(120±20)dwereselectedasexperimentalanimals.Usi replicate3×3Latinsquaredesign,thecowswererandomlydividedintothreegroups(n = 3pergroup).Twogroupsreceiveddifferentconcentrateformulationsbutthesamecornstrawroughageataconcen to−roughageratioof6 : 4(designatedCSAandCSBgroups),whiletheremaininggroupreceivedamixedroughag plantcornsilageataconcentrate−to−roughageratioof4 : 6(MFgroup).The84− dayexperimentconsistedofthree28 − dayperiods,eachincludinga21 − daypreliminaryperiodanda7−dayformalsamplingperiod.Theresultsshowedthat : (1)Nosignificantdifferenceswereobservedinmilkyieldormilkcompositionamonggroups(P > 0.05),buttheMFgroupexhibitedsignificantlyhigherlactationefficiencycomparedtoCSAandCSBgroups(P < 0.05).(2)DietaryintakeofC16 : 0andC18 : 0didnotdiffersignificantlyamonggroups(P > 0.05),whileC18 : 1c9intakeintheMFgroupwassignificantlylowerthaninCSAandCSBgroups(P < 0.05).(3)TheMFgroupshowedhighercontentsoflong−chainfattyacidsandtotalfattyacidsinarterialplasmath 3n3content(P < 0.05).(4)MammaryuptakeofC18 : 3n3(dailyaverage)inMFandCSBgroupswassignificantly 0.05),whilemammaryuptakeofotherlong−chainfattyacidsshowednosignificantdifferencesamonggroups(P 0.05).(5)Thesedifferenceswereultimatelyreflectedinmilkcomposition,withC18 : 3n3yieldintheMFgroupshowinganincreasingtrendcomparedtoCSAandCSBgroups(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). In conclusion, diets with different concentrate formulations but identical roughage sources did not significantly affect production performance of lactating dairy cows. However, high-quality roughage combinations can 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

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Milk fat is a crucial energy component in milk and one of the primary nutrients that enhance bodily functions and maintain sensory characteristics of dairy products [1]. Due to its substantial economic and health value, increasing attention has been directed toward improving milk fat percentage and composition. Numerous studies have demonstrated that dietary factors are the most direct determinants affecting milk fat content and composition, though ruminants present a unique case where diet influences the composition of milk fat synthesis precursors entering the bloodstream by altering rumen microbial communities and bacterial populations [1]. Liu [2] reported that feeding dairy cows diets with varying carbohydrate compositions changed ruminal acetate-to-propionate ratios, thereby affecting milk fat percentage. Roughage quality can influence dietary energy levels, with milk protein content increasing as roughage quality improves [3]. Zhang et al. [4] found that abomasal infusion of amino acids in lactating cows fed total mixed rations (TMR) with corn straw as the sole roughage had no significant effect on long-chain fatty acid content. Approximately half of the C16 and >C16 fatty acids in ruminant milk fat originate from blood lipids [5]. This study examined milk fat composition in dairy cows through the pathway of "diet type → ruminal microbial hydrogenation → bypass fat and fatty acid precursors in blood," aiming to investigate the uptake and utilization patterns of milk fat precursors under different dietary conditions.

1.1 Experimental Design and Animal Management

Nine healthy multiparous Holstein cows (2-3 parities) with body weight of (617$±21)kgandlactationdaysof(120±20)dwereselected.Theexperimentemployeda3−replicate3×$3 Latin square design, with cows randomly assigned to three groups (n=3 per group). Two groups received different concentrate formulations with corn straw as the common roughage at a concentrate-to-roughage ratio of 6:4 (designated CSA and CSB groups), while the remaining group received Chinese wildrye, alfalfa hay, and corn silage as mixed roughage at a concentrate-to-roughage ratio of 4:6 (MF group). Dietary nutrient levels met NRC (2001) requirements, with composition and nutrient levels shown in Table 1 . The 84-day experiment comprised three periods of 28 days each, including a 21-day preliminary period and a 7-day formal sampling period.

The trial was conducted at the Bingzhouhai Dairy Cooperative Demonstration Ranch of Inner Mongolia Dairy Union Technology Co., Ltd. Each cow was housed individually with access to an independent exercise area. Total mixed rations (TMR) were fed, with individual feed intake and refusals recorded daily. Cows were milked using a Lely portable mobile milking machine. Milking equipment, cow beds, and exercise areas were cleaned and disinfected regularly. Cows were fed twice daily at 07:00 and 19:00, with ad libitum access to water, and milked twice daily at 06:00 and 18:00.

1.2 Sample Collection

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

Blood samples were collected within two days after milk sampling (days 4-5 of the formal period). On day 1, fasting blood was collected twice: before morning feeding and before afternoon feeding, with caudal artery and mammary vein blood collected using anticoagulant tubes to prepare plasma, which was stored at -20 °C. On day 2, 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.

1.3.1 Calculation of Mammary Blood Flow, Precursor Uptake Rate, and Mammary Uptake in Lactating Cows

Mammary blood flow was estimated using C18:0 + C18:1 c9 as an endogenous marker, 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 a precursor) × Blood flow

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

1.3.2 Milk Composition Determination

Milk protein, 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 plasma was placed in a 10 mL centrifuge tube, mixed with 5 mL n-hexane-isopropanol solution (V/V=3/2), and vortexed for 2 min. (2) The upper organic phase (collected as completely as possible) was transferred to a hydrolysis tube, dried under nitrogen, then 0.5 mL n-hexane and 1 mL 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 sealed, and the mixture was esterified in a 90 °C water bath for 2 h. (5) After cooling to room temperature, 3 mL water and 5 mL n-hexane were added, shaken, and allowed to separate into layers. (6) The upper layer (collected as completely as possible) was dried under nitrogen to near dryness, reconstituted to 1 mL with n-hexane, vortexed for 30 s, and approximately 0.5 g anhydrous sodium sulfate was added to absorb water before instrumental analysis. (Note: All reagents used were chromatographic grade, and water used was ultra-pure. Complete transfer during pipetting was ensured for accurate results.)

1.3.3.2 Milk Sample Preparation

The procedure was as follows: (1) 2 mL milk sample was mixed with 4 mL n-hexane-isopropanol solution (V/V=3/2) and 2 mL sodium sulfate solution (6.67 g anhydrous sodium sulfate dissolved in 100 mL water), then 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. (3) 2 mL sodium hydroxide methanol solution (prepared as in section 1.3.3.1) was added, and the mixture was incubated in a 50 °C water bath for 15 min. After cooling, hydrochloric acid methanol solution was added and the mixture was incubated in an 80 °C water bath for 90 min. (4) After cooling to room temperature, 3 mL water and 6 mL n-hexane were added, shaken, and allowed to separate into layers. (5) The upper layer was collected as completely as possible, reconstituted to 10 mL with n-hexane (Note: due to high fatty acid content in milk, dilution to 10 mL represents a 5-fold dilution), approximately 2 g anhydrous sodium sulfate was added, and the supernatant was collected 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 µm pore size); column temperature was held at 120 °C for 10 min, then increased at 3.2 °C/min to 230 °C and held 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 Fat-Corrected 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 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 for experimental cows and fixed effects for period and treatment. Results are presented as least squares means. Significance level was set at $P<0.05$, with Tukey' s test used for multiple comparisons.

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 ($P>0.05$). The MF group exhibited significantly lower dry matter intake compared to CSA and CSB groups ($P<0.05$). No significant differences were observed among the three groups in milk yield or fat-corrected milk yield ($P>0.05$). However, the MF group showed significantly higher normal milk lactation efficiency and fat-corrected milk lactation efficiency compared to 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 did not differ significantly among groups ($P>0.05$).

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 ($P>0.05$).

2.3.1 Dietary Fatty Acid Composition and Intake

As shown in Table 4 , dietary contents of C16:0 and C18:0 did not differ significantly among the three groups ($P>0.05$), and cows' intake of these two fatty acids also showed no significant differences ($P>0.05$). Dietary C18:1 c9 content in CSA and CSB groups tended to be higher than in the MF group ($0.05\leq P<0.10$). Dietary C18:1 c9 intake in CSA and CSB groups was significantly higher than in the MF group ($P<0.05$). Dietary C18:2 n6 content and intake did not differ significantly among the three groups ($P>0.05$). Dietary C18:3 n3 content in the MF group was significantly higher than in 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 Long-Chain Fatty Acid Contents in Arterial and Venous Plasma

As shown in Table 5 , changes in fatty acid contents in arterial and venous plasma generally mirrored dietary fatty acid composition and intake. In the MF group, C18:3 n3 content in both arterial and venous plasma was significantly higher than in CSA and CSB groups ($P<0.05$).

2.3.3 Mammary Extraction Rate and Uptake of Fatty Acids

As shown in Table 6 , the MF group showed slightly higher extraction rates for total long-chain fatty acids compared to CSA and CSB groups, though the difference was not significant ($P>0.05$). No significant differences were observed among groups in mammary extraction rates for individual long-chain fatty acids ($P>0.05$). Regarding mammary uptake, C18:3 n3 uptake in CSB and MF groups tended to be significantly higher than in the CSA group ($0.05\leq P<0.10$).

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 CSA and CSB groups, with significant differences observed 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 CSA and CSB groups ($P<0.05$), while C18:2 n6 and polyunsaturated fatty acid contents and yields were significantly lower in the MF group ($P<0.05$).

2.3.5 Long-Chain Fatty Acid Flux

Dietary C18:1 c9 content in CSA and CSB groups was significantly higher than in the MF group ($P<0.05$), while C18:3 n3 content was significantly lower ($P<0.05$) (Table 4). Converting mammary uptake and milk fatty acid yield to daily averages (Table 9) revealed that mammary C18:3 n3 uptake in 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 showed an increasing trend compared to CSA and CSB groups ($0.05\leq P<0.10$), while C18:2 n6 yield was significantly lower ($P<0.05$).

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

The results indicated that dry matter intake was significantly lower in the MF group compared to CSA and CSB groups. The selected cows in this experiment produced approximately 20 kg of milk daily. The lower dry matter intake in the MF group may be attributed to the satiety effect of high roughage proportion. Additionally, this effect may be largely attributed to whole cottonseed supplementation 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 graded increases in whole cottonseed content and dry matter intake due to the extremely high energy level of whole cottonseed elevating net energy for lactation (NEL) intake.

While no significant differences were observed in milk yield or fat-corrected milk yield among groups, the MF group exhibited significantly higher normal milk lactation efficiency and fat-corrected milk lactation efficiency compared to 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 similar dietary crude protein and net energy for lactation intake, different roughage qualities had no effect on mammary lipogenic and proteinogenic capacity.

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 capacity for exogenous fatty acid conversion, thereby increasing milk fat percentage and reducing milk yield, 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] reported linear increases in milk yield and dry matter intake in primiparous cows when the concentrate-to-roughage ratio was increased from 25:75 to 45:55 and then to 65:35. However, this experiment showed significantly reduced dry matter intake in the MF group, possibly due to: (1) whole cottonseed in the MF group providing higher net energy for lactation as a high-quality bypass fat, thereby reducing dry matter intake; (2) the high roughage proportion in the MF group inducing satiety and reducing intake; and (3) in dairy goat studies, mixed roughage groups showed significantly improved milk protein and fat percentages compared to corn straw groups, likely due to alfalfa meal and corn silage supplementation when concentrate levels were equal between groups [18]. When dietary nutrient levels are low, high-quality roughage combinations may increase dry matter intake and improve milk yield, protein percentage, and fat percentage, but this effect diminishes when nutrient levels are increased. The reduced dry matter intake observed in this experiment may be attributed to satiety from high roughage proportion or feed selectivity. Overall, high-quality roughage combinations improve lactation efficiency in ruminants.

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

Measurement of mammary blood flow is fundamental to understanding mammary uptake and utilization of milk precursors [19]. Based on a hematocrit of 1/3, the ratio of mammary blood flow to milk yield in this experiment did not reach 500, deviating from the results of Annison et al. [20]. Long-chain fatty acids in blood primarily originate from dietary conversion, but ruminal microbial hydrogenation and other factors may destabilize blood fatty acid content and composition, causing 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 did not significantly affect mammary blood flow.

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 is the sole pathway for mammary blood supply, and its milk fat precursor composition is directly regulated by diet. This experiment showed that different diets did not significantly affect fatty acid composition in the external pudendal artery plasma but significantly impacted fatty acid composition and yield in the mammary gland. Shingfield et al. [21] proposed that fatty acid transport efficiency or blood flow could affect fatty acid supply and mammary uptake, leading to changes in milk fat composition. Yang et al. [22] found that mammary blood flow and blood fatty acid content influence mammary fatty acid uptake. Within certain ranges, increasing dietary or blood fatty acid content enhances mammary extraction rate, but excessively high levels reduce extraction efficiency [23]. This study demonstrated that dietary C18:3 n3 content and intake were significantly higher in the MF group than in CSA and CSB groups, while dietary C18:1 c9 content was significantly higher in CSA and CSB groups. Plasma analysis revealed that pre-mammary blood fatty acid composition generally reflected dietary fatty acid composition, indicating that dietary nutrients directly affect mammary uptake and utilization of milk fat precursors. The results showed minimal differences among groups in medium- and short-chain fatty acid content and yield in milk. Recent research has increasingly focused on health effects of medium- and short-chain saturated fatty acids, as several fatty acids with fewer than 10 carbons have been shown to positively regulate gene function, exhibit antiviral activity, and play roles in 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 genetic network regulation, milk fatty acid composition is substantially influenced by genetics, rumen function, and hormonal levels. High-quality roughage combinations may better regulate milk fat percentage and composition; for instance, studies have shown that higher dietary ratios of monounsaturated to polyunsaturated fatty acids provide greater protection against atherosclerosis and cardiovascular disease than diets rich in polyunsaturated fatty acids alone [24-25]. The milk fatty acid composition in the MF group supported this finding. With scientific advancement, milk fat research should move beyond simply increasing specific fatty acids toward optimizing milk fat composition through dietary regulation along the pathway of diet → rumen fermentation → intestinal absorption → hepatic conversion → blood regulation, directing ruminant milk fat composition toward profiles more beneficial for human health. Different diets did not significantly affect fatty acid composition in the external pudendal artery blood, though this pathway directly delivers diet-regulated milk fat precursors to the mammary gland.

In summary: (1) Under conditions of different concentrate-to-roughage ratios but similar nutrient levels, feeding lactating dairy cows diets with corn straw as the sole roughage versus high-quality mixed roughage showed no significant differences in milk protein percentage, milk fat percentage, or yields of milk protein and milk fat. (2) Different diets did not affect milk yield or mammary blood flow. (3) The composition of milk fat precursors circulating through the mammary gland was consistent with dietary composition, though original differences were diminished in mammary plasma. High-quality roughage combination diets promoted milk fat synthesis by enhancing mammary uptake of certain milk fat precursors.

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