

## Effects of External Pudendal Artery Infusion of Milk Fat Precursors on Milk Yield and Intramammary Short-Chain Fatty Acid Uptake Patterns in Dairy Cows (Postprint)

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### Abstract

This experiment investigated the effects of infusing milk fat precursors into the external pudendal artery on milk yield and the uptake patterns of short-chain fatty acids in the mammary gland of dairy cows. Eight healthy Holstein dairy cows were selected and divided into 2 groups, with 4 cows per group. A 2×2 crossover experimental design was adopted, with the two groups fed diets containing two different roughage to-roughage ratios of 45 : 55. The experiment consisted of 2 periods, each lasting 20 d, including a 14-d preliminary period, a 3-d vehicle infusion period, and a 3-d milk fat precursor infusion period. During the vehicle infusion period, the chain fatty acid concentrations and fatty acid proportions were determined. The results showed that : 1) Before milk fat precursor infusion, the CSC group had significantly lower milk yield,  $P < 0.10$ . 2) After milk fat precursor infusion, the CSF group had significantly higher milk fat production efficiency ( $P < 0.05$ ), and although the CSF group still had numerically lower milk yield,  $P < 0.10$ . 4) Except for a tendency for higher arteriovenous difference of butyrate in the CSF group compared with the CSC group, there were no significant differences in the uptake amount, uptake rate, and arteriovenous difference of acetate, propionate, butyrate, and short-chain fatty acids by the mammary gland between the CSC and MFC1 groups, CSC and CSF groups, or CSF and MFC2 groups ( $P > 0.05$ ). These results indicate that infusing milk fat precursors into dairy cows fed corn stover as roughage can significantly increase milk yield, milk fat percentage, and milk fat yield to levels similar to those of cows fed alfalfa as roughage, and with higher milk fat production efficiency, but has no significant effect on the uptake amount and uptake rate of short-chain fatty acids in the mammary gland.

## Full Text

# Effects of Infusing Milk Fat Precursors to External Pudic Artery on Milk Yield and Uptake Rule of Short-Chain Fatty Acids in Mammary Gland of Dairy Cows

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## Abstract

This experiment investigated the effects of infusing milk fat precursors to the external pudic artery on milk yield and the uptake rule of short-chain fatty acids in the mammary gland of dairy cows. Eight healthy Holstein cows were divided into two groups of four cows each, and a 2×2 cross-over experimental design was adopted. Cows in the two groups were fed diets containing different roughage sources: corn stover (CS group) and alfalfa (MF group), with a concentrate-to-roughage ratio of 45:55.

The experiment consisted of two stages, each lasting 20 days, including a 14-day preliminary feeding period, a 3-day carrier infusion phase, and a 3-day milk fat precursor infusion phase. During the carrier infusion period, both the MF and CS groups received carrier infusion and were designated as the MFC1 and CSC groups, respectively. During the milk fat precursor infusion period, the MF group continued to receive carrier infusion (MFC2 group), while the CS group received milk fat precursor infusion (CSF group). Milk yield, dry matter intake, and milk composition were measured, and plasma from the caudal artery and internal mammary vein was collected to determine short-chain fatty acid concentrations and proportions.

The results showed that: (1) Before milk fat precursor infusion, the CSC group had significantly lower milk yield, 4% fat-corrected milk (FCM) yield, milk fat yield, milk protein yield, and milk fat producing efficiency compared to the MFC1 group ( $P < 0.05$ ), while the concentration of short-chain fatty acids and the ratio of short-chain to long-chain fatty acids in caudal artery plasma tended to be lower in the CSC group ( $0.05 \leq P < 0.10$ ). (2) After milk fat precursor infusion, the CSF group exhibited significantly higher milk fat producing efficiency and milk protein percentage than the CSC group ( $P < 0.05$ ). Although the CSF group still had numerically lower milk yield, 4% FCM yield, milk fat percentage, and milk protein percentage compared to the MFC2 group, these differences were not significant ( $P > 0.05$ ), while the CSF group had significantly higher milk fat producing efficiency than the MFC2 group ( $P < 0.05$ ). (3) Following milk fat precursor infusion, the concentrations of acetic acid, propionic acid, and total short-chain fatty acids in caudal artery plasma were significantly higher in the CSF group than in the CSC group ( $P < 0.05$ ), and the concentrations of acetic

acid, butyric acid, and short-chain fatty acids were significantly higher than those in the MFC2 group ( $P < 0.05$ ), though the short-chain to long-chain fatty acid ratio tended to be lower than in the MFC2 group ( $0.05 \leq P < 0.10$ ). (4) Except for a tendency toward higher arteriovenous difference of butyric acid in the CSF group compared to the CSC group ( $0.05 \leq P < 0.10$ ), no significant differences were observed in the uptake quantity, uptake rate, or arteriovenous difference of acetic acid, propionic acid, butyric acid, or total short-chain fatty acids between the CSC and MFC1 groups, CSC and CSF groups, or CSF and MFC2 groups ( $P > 0.05$ ).

These results indicate that infusing milk fat precursors to dairy cows fed corn stover as roughage can significantly increase milk yield, milk fat percentage, and milk fat yield to levels similar to those achieved with alfalfa-based diets, while also improving milk fat producing efficiency. However, this treatment did not significantly affect the uptake quantity or uptake rate of short-chain fatty acids in the mammary gland.

**Key words:** mammary gland; external pudic artery; milk fat precursor; short-chain fatty acid

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## Introduction

Improving milk nutritional quality is essential for enhancing the market competitiveness of dairy products. Milk fat, a primary component of milk, serves as a crucial indicator of milk quality. Approximately 50% of fatty acids in milk are synthesized *de novo* in the mammary gland from milk fat precursors, including medium- and short-chain fatty acids (C4–C14) and 50% of C16 fatty acids, while C18 fatty acids and the remaining 50% of C16 fatty acids originate from the diet. China produces approximately 220 million tons of corn stover annually, making it a major agricultural residue. However, in regions where corn stover serves as the primary roughage for dairy cows, its nutritional value is substantially lower than that of high-quality forage, limiting the full expression of milk production performance and milk quality. Therefore, identifying methods to increase milk yield and improve milk quality while utilizing corn stover resources represents an urgent challenge.

Numerous studies have reported that infusing milk fat precursors promotes milk fat synthesis and other milk components in dairy cows, though results remain inconsistent. Zhang Fuquan investigated the effects of infusing fatty acids and amino acids into the external pudic artery of dairy cows on mammary fatty acid metabolism, finding that fatty acid infusion increased milk fat percentage and significantly enhanced mammary uptake of C18:2 n-6 under low-quality roughage conditions, while also increasing mammary uptake of C16:0 and improving mammary fatty acid balance compared to carrier infusion. Maxin et al. observed that ruminal infusion of acetate increased milk fat percentage by 6.5% without significantly affecting milk fat yield. Purdie et al. reported that

external pudic artery infusion of sodium acetate significantly increased milk protein percentage and tended to increase milk fat percentage, despite a 123% increase in plasma acetate concentration and a 128% increase in mammary uptake rate, without significantly altering milk fat yield or mammary uptake of long-chain fatty acids (LCFA) and  $\beta$ -hydrobutyrate (BHBA).

Our previous comparative feeding trials using corn stover versus alfalfa as roughage demonstrated that feeding corn stover alone reduced milk fat yield due to insufficient supply of milk fat precursors in the mammary gland. We hypothesized that supplementing deficient milk fat precursors under corn stover-based feeding conditions could improve milk yield and mammary uptake of short-chain fatty acids, though research in this area remains scarce. Therefore, this study infused milk fat precursors into the external pudic artery of dairy cows fed corn stover, measuring arteriovenous plasma concentrations and proportions of short-chain fatty acids and mammary uptake quantities to investigate the effects on milk yield and mammary uptake patterns. The findings provide a scientific basis for improving the utilization efficiency of straw feed resources and enhancing milk quality.

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## 1. Materials and Methods

### 1.1 Experimental Design

This study employed a  $2 \times 2$  cross-over experimental design. Eight Chinese Holstein cows [multiparous, mid-lactation, healthy, good body condition, milkyield ( $20.17 \pm 1.28$ ) kg/d] were randomly divided into two groups of four cows each and fed diets containing different roughage sources: alfalfa (MF group) and corn stover (CS group). The MF diet included alfalfa, corn silage, and Chinese wildrye as roughage sources, while the CS diet contained only corn stover. The experiment comprised two stages, each lasting 20 days, with a 14-day preliminary feeding period, a 3-day carrier infusion phase, and a 3-day milk fat precursor infusion phase. During the carrier infusion period, the MF group received carrier infusion (MFC1 group) and the CS group also received carrier infusion (CSC group). During the milk fat precursor infusion period, the MF group continued to receive carrier infusion (MFC2 group), while the CS group received milk fat precursor infusion (CSF group).

All groups received the same concentrate feed at a concentrate-to-roughage ratio of 45:55, delivered as a total mixed ration (TMR). Cows had free access to water and feed, with daily refusals maintained at 5% of the amount offered. Milking occurred twice daily at 06:00 and 18:00. Blood catheters were surgically placed in the external pudic artery one week before the infusion period. The basal diet composition and nutrient levels are presented in Table 1.

## 1.2 Infusion Solution Composition and Preparation

The infusion quantity of milk fat precursors was determined using the following formula: Infusion quantity = (Total fatty acids supplied by plasma during milk fat synthesis in MF group - Total fatty acids supplied by plasma during milk fat synthesis in CS group)  $\times$  10%.

A micro-infusion pump was used to infuse the external pudic artery with blood. The total daily infusion of milk fat precursors per cow was approximately 280 g, comprising about 200 g of long-chain fatty acids and 80 g of short-chain fatty acids. The total infusion volume was 2 L, administered continuously over 6 hours daily, starting at morning feeding. The infused milk fat precursors consisted of long-chain fatty acids C16:0, C18:0, C18:1c9, C18:2c6, C18:3n3 and short-chain fatty acid acetate, at concentrations of 6.50%, 3.34%, 7.74%, 46.44%, 5.57%, and 30.21%, respectively. Long-chain fatty acid sources included linseed oil (primarily providing C18:3n3), safflower seed oil (primarily providing C16:0, C18:1c9, and C18:2c6), and stearic acid triglyceride (primarily providing C18:0). The short-chain fatty acid source was anhydrous sodium acetate (primarily providing acetate).

The carrier infusion solution was prepared by dissolving 6 g of soybean lecithin in physiological saline with heating and stirring. After cooling to room temperature, the volume was adjusted to 1 L, followed by high-pressure homogenization. The homogenized solution was filtered through four layers of gauze, autoclaved, and then dispensed into sealed containers.

The milk fat precursor infusion solution was prepared following the methods of Stamey Lanier et al. [8] and Liu Dachuan et al. [9]. Linseed oil (Hohhot Mengyuexiang Vegetable Oil Processing Factory), safflower seed oil (COFCO Tayuan Xinjiang Edible Safflower Seed Oil Co., Ltd.), stearic acid triglyceride (Shanghai Darui Fine Chemical Co., Ltd.), and anhydrous sodium acetate (Yongda Chemical Reagent Co., Ltd.) were mixed and heated with stirring. Approximately 15 g of soybean lecithin was weighed and placed in a beaker with physiological saline, heated and stirred until uniform. The two prepared mixtures were combined, brought to a final volume of 2 L with physiological saline, and homogenized using a magnetic stirrer and homogenizer. The final solution was bottled and autoclaved, with preparation occurring as close to use as possible.

## 1.3 Sample Collection and Preparation

Milk samples were collected during the last two days of both the carrier infusion period and the milk fat precursor infusion period. Morning and evening milk samples were pooled according to production proportion and aliquoted into 50 mL sterile centrifuge tubes for milk composition analysis.

Blood samples were collected during the last two days of the milk fat precursor infusion period. On day 2 of the infusion period, 20 mL of blood was collected

from the internal mammary vein and caudal artery before morning infusion (0 h) and before afternoon feeding (0 h) to separate plasma. On day 3, 20 mL blood samples were collected from the internal mammary vein and caudal artery at 1 h after morning infusion and 4 h after afternoon feeding, then plasma was separated and stored at -20 °C. After the experiment, plasma samples from the four time points over two consecutive days were pooled in equal volumes and stored at -20 °C for fatty acid concentration determination.

## 1.4 Measurements

**1.4.1 Milk Performance** Milk yield and dry matter intake (DMI) were recorded daily during the infusion period. Milk composition parameters, including milk fat percentage, milk fat yield, milk protein percentage, and milk protein yield, were determined using an automatic milk composition analyzer (MilkoScan™ Minor-Type-78110, Denmark). The 4% fat-corrected milk (FCM) yield was calculated. Milk fat producing efficiency was calculated as: Milk fat producing efficiency (%) = Milk fat yield / DMI × 100.

**1.4.2 Acetic, Propionic, and Butyric Acid Concentrations in Caudal Artery and Internal Mammary Vein Plasma** Concentrations of acetic, propionic, and butyric acids in plasma were determined using the internal standard method with crotonic acid as the internal standard, analyzed by gas chromatography (GC-2010, Shimadzu, Japan).

Fatty acid concentrations in caudal artery and internal mammary vein plasma were measured by gas chromatography using an SP™-2560 capillary column and flame ionization detector (FID), with C17:0 as the internal standard and fatty acid methyl ester standards as external standards. A two-stage temperature programming method was used for separation and detection, covering short-chain fatty acids (acetic, propionic, and butyric acids) and long-chain fatty acids (C14:0, C16:0, C18:0, C18:1c9, C18:2c6, and C18:3n3). The ratio of short-chain to long-chain fatty acids was calculated as: Short-chain fatty acids/Long-chain fatty acids = Short-chain fatty acid concentration (mmol/L) / Long-chain fatty acid concentration (mmol/L).

**1.4.3 Mammary Blood Flow, Short-Chain Fatty Acid Uptake Rate, and Uptake Quantity** Blood flow was estimated using C18:0 + C18:1c9 as an endogenous indicator, following the method of Annison et al. [10]. Mammary short-chain fatty acid uptake rate and uptake quantity were calculated according to Enjalbert et al. [11] using the following formulas:

Blood flow (L/L) = Concentration of indicator in milk (mg/L) / [Concentration of indicator in caudal artery plasma (mg/L) - Concentration of indicator in internal mammary vein plasma (mg/L)]

Uptake rate (%) = 100 × Arteriovenous concentration difference (mg/L) / Arterial concentration (mg/L)

Uptake quantity (g/L) = Arteriovenous concentration difference (mg/L) × Blood flow × 1,000

Where the unit g/L for uptake quantity represents the mass of milk fat precursors taken up by the mammary gland per liter of milk produced.

### 1.5 Data Processing

Data were analyzed using the MIXED model in SAS 9.0 software. Differences were considered significant at  $P < 0.05$  and tendencies at  $0.05 \leq P < 0.10$ .

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## 2. Results

### 2.1 Effects of Milk Fat Precursor Infusion on Milk Performance

As shown in Table 2, before milk fat precursor infusion, the CSC group had significantly lower milk yield, 4% FCM yield, milk fat yield, milk protein yield, and milk fat producing efficiency compared to the MFC1 group ( $P < 0.05$ ). After milk fat precursor infusion, the CSF group showed a tendency for higher milk fat percentage ( $0.05 \leq P < 0.10$ ) and significantly higher milk fat producing efficiency and milk protein percentage than the CSC group ( $P < 0.05$ ). Although the CSF group remained numerically lower than the MFC2 group in milk yield, 4% FCM yield, and milk fat yield, these differences were not significant ( $P > 0.05$ ). However, the CSF group had significantly higher milk fat producing efficiency than the MFC2 group ( $P < 0.05$ ), and milk protein yield in the CSF group tended to be higher than in the MFC2 group ( $0.05 \leq P < 0.10$ ).

### 2.2 Effects of Milk Fat Precursor Infusion on Short-Chain Fatty Acid Concentrations and Fatty Acid Proportions in Plasma

As shown in Table 3 and Table 4, before milk fat precursor infusion, the CSC group tended to have lower short-chain fatty acid concentration and short-chain to long-chain fatty acid ratio in caudal artery plasma compared to the MFC1 group ( $0.05 \leq P < 0.10$ ). After infusion, the CSF group exhibited significantly higher concentrations of acetic acid, propionic acid, and total short-chain fatty acids in caudal artery plasma than the CSC group ( $P < 0.05$ ), with acetic acid, butyric acid, and short-chain fatty acid concentrations also significantly higher than in the MFC2 group ( $P < 0.05$ ). However, the short-chain to long-chain fatty acid ratio in the CSF group still tended to be lower than in the MFC2 group ( $0.05 \leq P < 0.10$ ). No significant differences were observed in short-chain fatty acid concentrations or fatty acid proportions in internal mammary vein plasma among any groups ( $P > 0.05$ ).

### 2.3 Effects of Milk Fat Precursor Infusion on Mammary Uptake of Short-Chain Fatty Acids

As shown in Table 5, except for a tendency toward higher arteriovenous difference of butyric acid in the CSF group compared to the CSC group ( $0.05 \leq P < 0.10$ ), no significant differences were found in uptake quantity, uptake rate, or arteriovenous difference of acetic acid, propionic acid, butyric acid, or total short-chain fatty acids between the CSC and MFC1 groups, CSC and CSF groups, or CSF and MFC2 groups ( $P > 0.05$ ). Blood flow in the CSC group tended to be significantly lower than in the MFC1 group ( $0.05 \leq P < 0.10$ ).

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## Discussion

Milk fat percentage is an important indicator of lactation performance in dairy cows. Research on the effects of external pudic artery infusion of milk fat precursors on milk performance remains limited, with most studies focusing on dietary supplementation or ruminal infusion of milk fat precursors and their effects on milk fat percentage and milk yield. Comparative studies examining precursor infusion under different dietary roughage regimes are particularly rare. Our results demonstrate that when both dietary groups received carrier infusion, the CSC group had significantly lower milk yield, 4% FCM yield, milk fat yield, and milk protein yield than the MFC1 group. After milk fat precursor infusion, although the CSF group remained numerically lower than the MFC2 group in milk yield, 4% FCM yield, and milk fat yield, these differences were not significant. Dry matter intake results showed no significant difference between the CSC and MFC1 groups during the carrier infusion period; however, after milk fat precursor infusion, the CSF group had significantly lower DMI than both the CSC and MFC2 groups. These findings suggest that milk fat precursor infusion significantly improved milk performance in corn stover-fed cows while reducing DMI, which partially explains the significantly increased milk fat producing efficiency.

Our previous research demonstrated significant differences in milk yield, milk fat percentage, and milk protein percentage among three dietary regimes, with both low-concentrate (concentrate-to-roughage ratio ~45:55) and high-concentrate (65:35) corn stover groups showing significantly lower milk yield and milk protein percentage than a mixed roughage group (alfalfa + corn silage + Chinese wildrye, ~45:55). Milk fat percentage was highest in the mixed roughage group and lowest in the low-concentrate corn stover group. Further investigation of mammary metabolism of milk fat precursors revealed significant differences in precursor concentrations and proportions entering the external pudic artery, as well as in mammary uptake quantities and proportions among dietary regimes. We hypothesized that reduced milk quality under corn stover feeding might be related to altered mammary uptake patterns of milk fat precursors, though relevant research remains scarce.

Ling confirmed that post-ruminal infusion of fatty acids improved milk yield, milk fat percentage, and milk fat yield in dairy cows, with linolenic acid showing more pronounced effects than other long-chain fatty acids. Relling et al. reported that feeding rumen-inert fats increased milk fat yield by 0.26 kg/d. Zhang Yubin et al. found that dietary supplementation with protected sunflower oil increased milk fat percentage. Zhang et al. observed that increased dietary and blood fatty acid supply reduced corresponding fatty acid uptake and transport efficiency but increased their concentration and yield in milk. Maxin et al. found that ruminal acetate infusion increased milk fat percentage by 6.5% without significantly affecting milk fat yield.

To further investigate why corn stover-fed cows had lower milk performance than alfalfa-fed cows, this study examined changes in short-chain fatty acid uptake patterns in caudal artery and internal mammary vein plasma before and after infusion. Before infusion, the CSC group had numerically lower concentrations of acetic, propionic, and butyric acids, as well as lower acetic/propionic acid ratio and (acetic+butyric)/propionic acid ratio in caudal artery plasma, with short-chain fatty acid concentration tending to be significantly lower than in the MFC1 group. After infusion, the CSF group showed significantly higher concentrations of acetic acid, propionic acid, and total short-chain fatty acids in caudal artery plasma than the CSC group, with acetic acid, butyric acid, and short-chain fatty acid concentrations also significantly higher than in the MFC2 group. These results indicate that milk fat precursor infusion increased the concentrations of milk fat precursors (acetic acid, propionic acid, butyric acid, and total short-chain fatty acids) in caudal artery blood of corn stover-fed cows, partially explaining why precursor infusion significantly increased milk yield, milk fat percentage, and milk fat yield to levels comparable to the alfalfa group. This effect appears related to the elevated concentrations of these precursors in caudal artery plasma after infusion, though specific mechanisms require further investigation.

Our results also showed that before infusion, the CSC group had significantly lower milk protein yield than the MFC1 group (25.42% reduction), and after infusion, the CSF group tended to have lower milk protein yield than the MFC2 group (24.59% reduction). This suggests that milk fat precursor infusion in corn stover-fed cows not only enhances milk fat synthesis and production performance but also improves milk protein synthesis, narrowing the gap with alfalfa-fed cows. This study did not examine the effects of individual acetate or long-chain fatty acid infusion, nor the impact of specific amino acid infusion on mammary fatty acid and amino acid uptake patterns. Future research should investigate these aspects to better understand the mechanisms underlying reduced milk performance in corn stover-fed cows from the perspective of precursor uptake patterns, thereby providing a scientific basis for improving straw utilization efficiency and milk quality.

## Conclusion

Infusing milk fat precursors to dairy cows fed corn stover as roughage significantly increased milk yield, milk fat percentage, and milk fat yield to levels comparable to those achieved with alfalfa-based diets, while improving milk fat producing efficiency. However, this treatment did not significantly affect the uptake quantity or uptake rate of short-chain fatty acids in the mammary gland.

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