Effect of External Pudendal Artery Infusion of Amino Acid Mixture on Intramammary Short-Chain Fatty Acid Uptake Patterns in Dairy Cows Fed Corn Straw-Based Diets: Postprint

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Abstract

This study employed arteriovenous cannulation technique and used mid-lactation Holstein dairy cows as experimental subjects to investigate the effects of external pudendal artery infusion of amino acid (AA) mixture on milk production performance, concentrations and proportions of short-chain fatty acids (SCFA) in caudal arterial and milk vein blood, and intramammary SCFA uptake dynamics in dairy cows fed corn stover diets. A 2×2 crossover experimental design was adopted, in which 8 multiparous (2nd–3rd parity) Holstein dairy cows with good body condition, similar body weight, and daily milk yield of (20.17±1.28) kg were randomly divided into 2 groups fed different diets [alfalfa group (MF group) and corn stover group (CS group)], with 4 cows per group. Both groups were fed diets with a concentrate-to-forage ratio of 45:55, with the same concentrate composition but different forage compositions; the forage in the MF group consisted of alfalfa hay, corn silage, and Chinese wildrye, while the CS group completely replaced the forage in the MF group diet with single corn stover. The experiment was divided into 2 periods, each lasting 20 d, which were further divided into a dietary adaptation period (preliminary period) of 14 d, a vehicle infusion period of 3 d, and a formal infusion period of 3 d. In Period 1, during the vehicle infusion period, cows in the MF group received vehicle infusion (positive control group 1), and cows in the CS group also received vehicle infusion (control group); during the formal infusion period, cows in the MF group continued to receive vehicle infusion (positive control group 2), while cows in the CS group received AA mixture infusion. In Period 2, the two groups of animals were switched and the treatment methods were the same as in Period 1. Milk and blood samples were collected on the last 2 d of each formal infusion period. The results showed that external pudendal artery infusion of AA mixture in the CS group significantly

increased milk protein percentage (P<0.05), and had certain promoting effects on milk yield, 4% fat-corrected milk (FCM) yield, milk fat percentage, milk fat yield, and milk protein yield, but some indicators remained significantly lower than those in the MF group (P<0.05). External pudendal artery infusion of AA mixture in the CS group tended to significantly increase acetate concentration in milk vein blood (P=0.09), significantly decreased arteriovenous difference of acetate in the mammary gland (P<0.05), and narrowed the gap between the CS and MF groups in terms of acetate uptake amount and uptake efficiency by the mammary gland. External pudendal artery infusion of AA mixture in the CS group had certain promoting effects on increasing acetate/propionate and (acetate+butyrate)/propionate ratios in caudal arterial blood (P>0.05). It was concluded that external pudendal artery infusion of AA mixture in dairy cows fed corn stover as forage could significantly increase milk protein percentage, increase acetate concentration in milk vein blood, and simultaneously narrow the gap with dairy cows fed alfalfa hay, corn silage, and Chinese wildrye as forage in terms of acetate uptake amount and uptake efficiency by the mammary gland.

Full Text

Effects of Infusing Amino Acid Mixture into External Pudendal Artery on Uptake Patterns of Short-Chain Fatty Acids in Mammary Gland of Dairy Cows Fed Corn Stover Diets

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Abstract

This study investigated the effects of infusing an amino acid (AA) mixture into the external pudendal artery on lactation performance, short-chain fatty acid (SCFA) concentrations and their ratios in caudal artery and mammary vein blood, and the uptake patterns of SCFAs in the mammary gland of mid-lactation Holstein cows fed corn stover diets, using arteriovenous catheterization techniques. A 2×2 crossover design was employed with eight healthy multiparous (2–3 parities) Holstein cows in good body condition, similar body weight, and with daily milk yields of (20.17±1.28) kg. The cows were randomly divided into two groups fed different diets [alfalfa group (MF group) and corn stover group (CS group)], with four cows per group. Both groups received diets with a forage-to-concentrate ratio of 45:55 and identical concentrate composition, but different forage sources. The MF group forage consisted of alfalfa hay, corn silage, and Chinese wildrye, while the CS group had all forage in the MF diet replaced entirely with corn stover. The experiment comprised two periods, each lasting 20 days and consisting of a 14-day dietary adaptation period (prelimi-

nary period), a 3-day carrier infusion phase, and a 3-day formal infusion phase. In the first period, during the carrier infusion phase, cows in the MF group received carrier infusion (positive control group 1) and cows in the CS group also received carrier infusion (control group). During the formal infusion phase, MF group cows continued to receive carrier infusion (positive control group 2), while CS group cows received AA mixture infusion. In the second period, the two groups were switched and processed using the same method as in the first period. Milk and blood samples were collected on the last two days of each formal infusion phase. The results showed that infusing the AA mixture into the external pudendal artery of CS group cows significantly increased milk protein percentage ($P<0.05$) and tended to improve milk yield, 4% fat-corrected milk (FCM) yield, milk fat percentage, milk fat yield, and milk protein yield, though some indices remained significantly lower than those in the MF group ($P<0.05$). The AA mixture infusion tended to increase acetic acid concentration in mammary vein blood ($P=0.09$), significantly reduced the arteriovenous difference of acetic acid in the mammary gland ($P<0.05$), and narrowed the gaps in acetic acid uptake quantity and uptake efficiency between CS and MF groups. The infusion also showed some promoting effects on increasing acetic acid/propionic acid and (acetic acid+butyric acid)/propionic acid ratios in caudal artery blood ($P>0.05$). In conclusion, infusing an AA mixture into the external pudendal artery of dairy cows fed corn stover as the sole forage can significantly improve milk protein percentage, increase acetic acid concentration in mammary vein blood, and reduce the gaps in acetic acid uptake quantity and uptake efficiency in the mammary gland compared with cows fed alfalfa hay, corn silage, and Chinese wildrye as mixed forages.

Keywords: dairy cows; external pudendal artery; amino acid mixture; short-chain fatty acids; uptake

Introduction

As living standards and health awareness improve, consumer demands for milk quality have also increased. Milk fat and protein content are important indicators of milk quality. China is a major producer of corn stover, which is abundant and widely available. However, due to limited high-quality forage resources, corn stover serves as the primary forage for dairy cows in some regions. Feeding diets based mainly on corn stover severely reduces milk yield, milk fat percentage, and milk protein percentage in dairy cows. Therefore, in-depth research on the causes of poor lactation performance and milk quality in cows fed stover-based diets, along with nutritional regulation technologies, holds important theoretical and practical significance for improving the utilization efficiency of stover resources in dairy production.

Some studies have found that amino acid (AA) mixtures not only regulate milk protein synthesis but also influence milk fat synthesis. However, most current

research focuses on dietary supplementation of rumen-protected protein (including AAs, small peptides, and non-protein nitrogen) and its effects on dairy cow performance. Systematic studies investigating the effects of AA mixture infusion on milk fat synthesis under corn stover dietary conditions have not been reported. Limited data are available on the effects of intravenous or external pudendal artery infusion of milk protein precursors on milk fat synthesis in dairy cows or dairy goats. Chamberlain et al. found that intravenous infusion of methionine in dairy cows increased milk fat yield. Duan Bin reported that infusing 6.2 g/d of AA mixture into the external pudendal artery of Guanzhong dairy goats increased mammary uptake of acetic acid by 7.2% and glucose uptake by 111.4%. Our preliminary research indicated that cows fed corn stover as the main forage had significantly lower milk yield and milk fat and protein percentages compared with cows fed mixed forages of alfalfa and corn silage. Significant differences were also observed in the supply of milk fat precursors in the external pudendal artery blood, suggesting that the inferior lactation performance and milk quality associated with corn stover diets might be related to differences in the concentration of exogenous milk fat precursors in caudal artery blood. However, systematic reports on this topic are currently lacking.

Therefore, this experiment utilized arteriovenous catheterization techniques to infuse an AA mixture into the external pudendal artery of cows fed corn stover diets, investigating its effects on SCFA concentrations and ratios in caudal artery and mammary vein blood and on SCFA uptake patterns in the mammary gland. This research provides a theoretical basis for scientifically explaining the main reasons for differences in lactation performance and milk quality between cows fed corn stover versus those fed alfalfa and corn silage mixed forages, and establishes a scientific foundation for effectively improving the utilization efficiency of stover feed resources and enhancing milk quality.

Materials and Methods

1.1 Experimental Animals and Design This experiment selected eight healthy, disease-free multiparous (2–3 parities) Chinese Holstein cows in mid-lactation with similar body weight and daily milk yields of (20.17±1.28) kg/d. A 2×2 crossover design was used, with cows randomly divided into two groups fed different diets: an alfalfa group (MF group) and a corn stover group (CS group), with four cows per group. Both groups received diets with a forage-to-concentrate ratio of 45:55 and identical concentrate composition, but different forage sources. The MF group forage consisted of alfalfa hay, corn silage, and Chinese wildrye, while the CS group had all forage in the MF diet completely replaced with corn stover.

The experiment comprised two periods, each lasting 20 days and including a 14-day dietary adaptation period (preliminary period), a 3-day carrier infusion phase, and a 3-day formal infusion phase. Both MF and CS groups were sub-

jected to carrier infusion (C) and AA mixture infusion (A). In the first period, during the carrier infusion phase, MF group cows received carrier infusion (positive control group 1, MFC1), and CS group cows also received carrier infusion (control group, CSC). During the formal infusion phase, MF group cows continued to receive carrier infusion (positive control group 2, MFC2), while CS group cows were infused with the AA mixture (CSA). In the second period, to eliminate individual differences, the two groups were switched and processed using the same method as in the first period.

During the experiment, cows were fed total mixed rations (TMR) twice daily (morning and evening), with free access to feed and water. Daily feed refusals were maintained at 5% of the amount offered, and cows were milked twice daily (morning and evening). The TMR composition and nutrient levels are shown in Table 1 .

1.2 Composition and Preparation of AA Mixture Infusate The carrier infusate was prepared by accurately weighing 6 g of soybean lecithin, dissolving it in physiological saline, and heating while stirring evenly. After the mixture cooled to room temperature, it was diluted to 1 L, subjected to high-pressure homogenization, filtered through four layers of gauze, autoclaved, and then bottled and sealed for later use.

The AA mixture infusion quantity was calculated as: (total AA supplied by plasma for milk protein synthesis in MF group cows - total AA supplied by plasma for milk protein synthesis in CS group cows) × 10%. This value served as the supplemental dose of AA mixture in the blood of CS group cows. The preparation of the AA mixture infusate referenced studies by Mepham and Chamberlain et al. The daily AA mixture infusion quantity per cow was approximately 152 g. The proportions of Thr, Ser, Glu, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, Lys, His, and Arg in the AA mixture were 12.50%, 5.92%, 11.18%, 7.24%, 11.84%, 1.32%, 17.76%, 1.32%, 5.26%, 6.58%, 2.63%, 3.29%, 5.92%, 3.29%, and 3.95%, respectively.

Blood catheters were surgically placed in the external pudendal artery one week before the carrier infusion period. A micro-infusion pump was used for blood infusion. The infusate was bottled and autoclaved before infusion. Infusion was performed for 6 hours daily, starting in the morning after feeding began. The total infusate volume was 2 L, with an AA mixture concentration of 76 g/L in the AA mixture infusate.

1.3 Collection and Preparation of Plasma and Milk Samples During each formal infusion period, caudal artery blood samples (20 mL) were collected at 0 h before morning infusion and at 0 h before afternoon feeding on day 2. Mammary vein blood samples (20 mL) were collected at 1 h after morning infusion and 4 h after afternoon feeding on day 3. Blood samples were anticoagulated with sodium heparin, centrifuged at 4,000×g for 10 min at 4 °C to prepare plasma, which was stored at -20 °C for later analysis.

During the last two days of each formal infusion period, milk samples were collected during milking while recording milk yield. Morning and evening milk samples were mixed according to yield proportions, then aliquoted into 50 mL sterile centrifuge tubes. One portion was used for immediate milk composition analysis, while the remainder was stored at -20 °C for later analysis.

1.4 Measurement Indicators and Methods Dietary neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using the filter bag technique on an ANKOM 200 fiber analyzer. Crude protein (CP) content was measured by the Kjeldahl method, crude fat (EE) content by Soxhlet extraction, and starch content by enzymatic hydrolysis on a Beckman Synchron CX4/Pro automatic biochemical analyzer. The specific procedures for these measurements followed the methods described in "Feed Analysis and Feed Quality Detection Technology."

Lactation performance indicators included milk fat percentage and yield, milk protein percentage and yield, milk fat production efficiency, and dry matter intake (DMI). Milk fat and protein percentages were determined using an automatic milk composition analyzer (MilkoScanTM Minor-Type 78110, FOSS Analytical A/S 69, DK-3400, Denmark). Daily feed intake was recorded during the experimental period to calculate DMI for each cow.

Milk fat yield (kg/d) = milk fat percentage × average milk yield.
Milk protein yield (kg/d) = milk protein percentage × average milk yield.
Milk fat production efficiency (%) = (milk fat yield/DMI) × 100.

Fatty acid concentrations in caudal artery and mammary vein blood were determined by gas chromatography (Shimadzu GC-2010, Japan). SCFA concentrations (including acetic acid, propionic acid, and butyric acid) were measured by internal standard method using crotonic acid as the internal standard. Long-chain fatty acids (C18:0, C18:1cis-9) were quantified using heptadecanoic acid as internal standard and fatty acid methyl ester standards as external standards, with separation by a two-stage temperature programming method. The ratios of acetic acid/propionic acid and (acetic acid+butyric acid)/propionic acid in caudal artery and mammary vein blood were also calculated.

Mammary blood flow was estimated using C18:0+C18:1cis-9 as an endogenous indicator. SCFA uptake efficiency and uptake quantity were calculated according to the method of Enjalbert et al.:

Blood flow (L/L milk) = content of C18:0+C18:1cis-9 in milk / (content of 18:0+18:1cis-9 in arterial blood - content of 18:0+18:1cis-9 in venous blood).

Uptake efficiency (%) = (arteriovenous concentration difference / arterial concentration) × 100.
Uptake quantity (mmol/L milk) = arteriovenous concentration difference × blood flow.

1.5 Data Processing Statistical analysis was performed using the MIXED model in SAS 9.0 software. Differences were considered significant at $P<0.05$ and tended to be significant at $0.05 P<0.10$.

Results

2.1 Effects of External Pudendal Artery AA Mixture Infusion on Lactation Performance As shown in Table 2 , before infusion, the CSC group had significantly lower DMI, milk yield, 4% FCM yield, milk fat yield, milk protein yield, and milk fat production efficiency compared with the MFC1 group ($P<0.05$). After infusion, the CSA group tended to have lower FCM yield than the MFC2 group ($P=0.06$), and all indices except milk fat percentage and milk protein percentage remained significantly lower than those in the MFC2 group ($P<0.05$). Compared with the CSC group, the CSA group showed significantly increased milk protein percentage ($P<0.05$), while other indices showed no significant changes ($P>0.05$).

2.2 Effects of External Pudendal Artery AA Mixture Infusion on Acetic Acid, Propionic Acid, and Butyric Acid Concentrations and Ratios in Caudal Artery and Mammary Vein Blood As shown in Table 3 , no significant differences were observed in acetic acid, propionic acid, butyric acid, or total SCFA concentrations in caudal artery and mammary vein blood between the CSC and MFC1 groups or between the CSA and MFC2 groups ($P>0.05$). The CSA group tended to have higher acetic acid concentration in mammary vein blood compared with the CSC group ($P=0.09$), while other indices showed no significant differences between these two groups ($P>0.05$).

As shown in Table 4 , AA mixture infusion had no significant effects on acetic acid/propionic acid or (acetic acid+butyric acid)/propionic acid ratios in caudal artery and mammary vein blood ($P>0.05$).

2.3 Effects of External Pudendal Artery AA Mixture Infusion on SCFA Uptake Patterns in Mammary Gland As shown in Table 5 , the CSA group had significantly higher blood flow than the MFC2 group ($P<0.05$), and tended to have lower arteriovenous difference of acetic acid than the MFC2 group ($P=0.05$). No significant differences were observed in arteriovenous differences, uptake efficiencies, or uptake quantities of acetic acid, propionic acid, butyric acid, or total SCFAs between the CSC and MFC1 groups, between CSC and CSA groups, or between the CSA and MFC2 groups ($P>0.05$).

Discussion

Although corn stover is widely used as a primary forage for dairy cows in many regions due to its abundant availability and limited high-quality forage resources,

its low nutritional value restricts lactation performance. Therefore, investigating the causes of poor lactation performance in cows fed corn stover and developing improvement measures holds important theoretical and practical significance for enhancing the utilization efficiency of corn stover in dairy production.

Our preliminary research indicated that cows fed corn stover as the main forage had significantly lower milk yield, milk fat percentage, and milk protein percentage compared with cows fed mixed forages of alfalfa and corn silage. This difference might be related to variations in the concentration of exogenous milk fat precursors supplied in caudal artery blood. However, systematic studies on this topic are currently lacking. The present study demonstrated that external pudendal artery infusion of an AA mixture significantly improved milk protein percentage in cows fed corn stover diets.

Before AA mixture infusion, the CSC group had significantly lower milk yield, FCM yield, milk fat production efficiency, and milk protein yield compared with the MFC1 group, with these indices representing 62.70%, 67.90%, 35.34%, and 48.57% of the MFC1 group values, respectively. After AA mixture infusion, although the CSA group remained significantly lower than the MFC2 group in these indices, the gaps narrowed, with CSA group values representing 70.86%, 75.23%, 70.93%, and 66.10% of the MFC2 group values, respectively. These results indicate that while lactation performance in corn stover-fed cows remained lower than that of cows fed alfalfa, corn silage, and Chinese wildrye after AA mixture infusion, performance was improved to varying degrees, narrowing the gap between the two groups. This suggests that AA mixtures can regulate not only milk protein synthesis but also milk fat synthesis. However, despite effectively compensating for nutritional deficiencies caused by feeding corn stover as the sole forage and improving lactation performance, gaps remained compared with feeding alfalfa, corn silage, and Chinese wildrye. Therefore, further investigation is needed to determine how nutritional regulation technologies can further improve the conversion efficiency of corn stover feed resources and dairy cow performance.

Baumrucker identified three key factors for mammary gland utilization of nutrients: nutrient concentration in mammary blood, blood flow, and nutrient transport efficiency. With the application of arteriovenous catheterization techniques, recent studies have found that arteriovenous concentration differences of most AAs in the mammary gland correlate linearly with arterial blood concentrations. Duan Bin reported that infusing 6.2 g/d of AA mixture into the external pudendal artery of Guanzhong dairy goats increased mammary uptake of acetic acid by 7.2%. Wang Qiang found that infusing a certain amount of AA mixture into the external pudendal artery of Guanzhong dairy goats significantly increased concentrations of acetic acid, butyric acid, and total volatile fatty acids in mammary plasma, as well as significantly increasing mammary uptake of acetic acid. Additionally, mammary uptake of milk fat precursors increased by 50.0%–71.5%, and uptake of lactose precursors increased by 9.1%–30.6%. These findings suggest that AA mixture infusion can improve mammary

uptake of milk fat precursors such as acetic acid and butyric acid in dairy goats and cows.

To further explore why lactation performance was lower in CS group cows compared with MF group cows, this study examined changes in SCFA concentrations in caudal artery and mammary vein blood and SCFA uptake patterns in the mammary gland before and after AA mixture infusion. The results showed that AA mixture infusion increased acetic acid concentration in mammary vein blood, tended to increase the acetic acid/propionic acid ratio, and narrowed the gaps in acetic acid uptake quantity and uptake efficiency between CS and MF groups. Before infusion, CSC group acetic acid uptake quantity and efficiency represented 55.3% and 58.1% of MFC1 group values, respectively. After infusion, CSA group acetic acid uptake quantity and efficiency represented 89.4% and 61.0% of MFC2 group values, respectively. This may be one reason for the improved lactation performance, though systematic reports on this mechanism are currently lacking and require further investigation.

Changes in blood concentration of milk fat synthesis substrates and blood flow are primary factors affecting mammary uptake efficiency. This study found that after AA mixture infusion, dairy cow blood flow increased and the arteriovenous difference of acetic acid increased, which may be one reason for the enhanced mammary uptake quantity and efficiency of acetic acid. Safayi and Nielsen reported that essential amino acid (EAA) supplementation could promote entry of acetic acid and -hydroxybutyric acid into the mammary gland, possibly due to metabolic needs and assistance in de novo fatty acid synthesis. Purdie et al. reported that extra-arterial infusion of AAs in dairy cows increased arterial plasma insulin concentration, promoting mammary uptake of energy metabolites glucose and acetic acid. Currently, relevant research reports are scarce, and the mechanisms require further exploration.

Furthermore, many studies have confirmed that AA mixture infusion affects mammary uptake patterns of AAs. Sun Manji et al. observed that infusing 49.2 and 65.6 g/d of AAs into the external pudendal artery of Guanzhong dairy goats significantly increased concentrations of most AAs in mammary arterial blood and mammary uptake of most AAs, though no significant effects were observed on concentration and uptake of lysine (Lys), valine (Val), and leucine (Leu). Mammary intake of EAAs increased by 15.4% and 16.1%, respectively, while intake of non-essential amino acids (NEAAs) increased by 31.3% and 29.4%, respectively. Liu Fei infused total AAs (group F), Lys-deficient total AAs (group -L), and no-AA carrier (group O) into the abomasum of lactating goats under basal dietary conditions and found that milk protein yield in group F was significantly higher than in groups -L and O. These findings suggest that the improved lactation performance in CS group cows after AA mixture infusion may be related to altered mammary uptake patterns of the AA mixture, warranting further in-depth investigation from this perspective.

Although this study provides a scientific basis for effectively improving dairy cow utilization efficiency of corn stover feed resources and enhancing milk quality,

the small number of experimental animals necessitates further verification of these results.

Conclusion

External pudendal artery infusion of an AA mixture in corn stover-fed dairy cows improved milk yield, FCM yield, milk fat percentage, milk fat yield, and milk protein yield, though these indices remained lower than those in the alfalfa group, the gaps were narrowed. The infusion significantly increased milk protein percentage, enhanced mammary blood flow and acetic acid concentration in mammary vein blood, and reduced the gaps in mammary acetic acid uptake quantity and uptake efficiency between CS and MF groups.

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