# Effects of Jugular Infusion of Arginine on Lactation Performance and Casein Synthesis in Mid-Lactation Dairy Cows (Postprint)

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## Abstract

The objective of this study was to investigate the effects of jugular arginine infusion on lactation performance and milk casein synthesis in mid-lactation dairy cows. Six Holstein dairy cows with similar body weight, parity, lactation stage, milk yield, and body condition were randomly divided into 3 groups (2 cows per group): casein pattern group (control group), arginine infusion group, and alanine isonitrogenous group (isonitrogenous with the arginine infusion group). A 3$×$3 replicated Latin square design was adopted, with each period lasting 22 days (7-day infusion period + 15-day interval period). Lactation performance, casein content, and casein gene expression were measured. The results showed: 1) On day 5 of infusion, the arginine infusion group had significantly higher milk protein and milk solids-not-fat contents than the casein pattern group ($P<0.05$); on day 6, the arginine infusion group had significantly higher milk fat percentage than the alanine isonitrogenous group ($P<0.05$). 2) The $\alpha$-casein content in the casein pattern group was significantly lower than in the other two groups ($P<0.05$); $\beta$-casein content showed no significant differences among the three groups ($P>0.05$);  -casein content was highest in the arginine infusion group, significantly higher than the other two groups ($P<0.05$). 3) The expression levels of $\alpha$s1-casein gene (CSN1S1) and $\alpha$s2-casein gene (CSN1S2) in the arginine infusion group were significantly higher than in the other two groups ($P<0.05$). In conclusion, arginine infusion increased the contents of $\alpha$-casein and  -casein in milk protein, as well as the expression levels of CSN1S1 and CSN1S2 in dairy cow mammary tissue, which is beneficial for improving milk protein percentage and milk quality.

## Full Text

# Effects of Arginine Infusion through Jugular Vein on Lactation Performance and Casein Synthesis in Mid-Lactation Dairy Cows

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## Abstract

This study investigated the effects of arginine infusion through the jugular vein on lactation performance and casein synthesis in mid-lactation dairy cows. Six Holstein cows with similar body weight, parity, lactation stage, milk yield, and body condition were randomly allocated into three groups (two cows per group) in a 3$×$3 Latin square design: a casein model group (control), an arginine infusion group, and an alanine iso-nitrogen group (equal nitrogen to the arginine group). Each experimental period lasted 22 days, comprising a 7-day infusion phase followed by a 15-day interval. Milk performance, casein content, and casein gene expression were measured. The results showed that: (1) On day 5 of infusion, the arginine group exhibited significantly higher milk protein and nonfat milk solids contents compared to the casein model group ($P<0.05$); on day 6, the arginine group showed significantly higher milk fat percentage than the alanine iso-nitrogen group ($P<0.05$). (2) The casein model group had significantly lower $\alpha$-casein content than the other two groups ($P<0.05$); $\beta$-casein content did not differ significantly among the three groups ($P>0.05$);  -casein content was highest in the arginine group, significantly exceeding the other two groups ($P<0.05$). (3) The arginine group demonstrated significantly higher expression levels of $\alpha$s1-casein gene (CSN1S1) and $\alpha$s2-casein gene (CSN1S2) compared to the other groups ($P<0.05$). In conclusion, arginine infusion increased $\alpha$-casein and  -casein contents in milk, as well as CSN1S1 and CSN1S2 expression levels in mammary tissue, thereby contributing to improved milk protein percentage and quality.

**Keywords:** jugular vein infusion; arginine; lactation performance; casein

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As one of the most important and high-quality nutrient sources and functional foods for humans, milk not only provides essential amino acids and fatty acids but also contains casein as its predominant protein component (over 80% of total milk protein), which possesses various biological functions and health benefits. Casein can bind mineral elements, particularly calcium ions, forming soluble complexes that enhance mineral absorption, and contains multiple functional peptides. Moreover, casein is uniquely synthesized by mammary tissue. Therefore, investigating the regulatory mechanisms and technologies of casein synthesis in dairy cow mammary glands represents a crucial approach for improving milk protein percentage and quality.

Previous studies have demonstrated that arginine promotes epithelial cell proliferation and protein synthesis. Supplementing culture medium with 0.3 mmol/L arginine benefits intestinal epithelial cell proliferation and protein turnover while alleviating endotoxin-induced apoptosis. Adding 556.00 mg/L arginine to culture medium promotes bovine mammary epithelial cell proliferation and enhances casein gene expression through the mammalian target of rapamycin (mTOR) signaling pathway, thereby stimulating casein synthesis. Furthermore, providing Wistar rats with double their arginine requirement promoted mammary alveolar development and increased casein synthesis. These findings indicate that arginine can enhance casein synthesis in both in vitro cultured mammary epithelial cells and rodent models. Bovine studies have also shown that abomasal arginine infusion increased milk protein yield compared to control groups, though without analyzing casein composition changes. Consequently, the precise mechanisms and patterns of arginine’ s effects on milk protein (casein) synthesis remain incompletely understood. This study employed jugular vein infusion to administer an optimal arginine dose (based on in vitro studies) to mid-lactation cows, measuring lactation performance, milk quality, dynamic casein content changes, and mammary casein gene expression to elucidate arginine’ s regulatory patterns and mechanisms in casein synthesis, providing fundamental data for milk quality modulation technologies.

### 1.1 Experimental Animals and Design

Six Holstein cows with consistent body weight, parity (4th lactation), lactation stage [(80$±2)*days*], *milkyield*[(21.0±$1.0) kg], and body condition score (3.0) were selected from the Yangzhou University experimental farm. The basal diet was formulated according to NRC (2001) nutrient requirements for dairy cattle and provided throughout the experimental period. Diet composition and nutrient levels are presented in Table 1 , with Chinese wild rye fed separately and other ingredients provided as total mixed ration. Nutrient composition of the basal diet was determined according to Zhang Liying. During the preliminary period, individual feed intake was recorded over 15 days to calculate feeding amounts.

Cows were housed individually in the same barn, fed equal amounts twice daily (Chinese wild rye first, followed by mixed concentrate), with orts collected to calculate dry matter intake (20.21, 18.85, and 19.12 kg/d for the three groups, respectively; $P>0.05$). Milk was collected three times daily, with free access to water. An indwelling catheter was installed in the left jugular vein and flushed twice daily with sterile heparinized saline (750 IU/mL). Cows were randomly divided into three treatment groups: (1) casein model infusion group (control, receiving amino acid mixture supplemented to achieve casein pattern based on measured external pudendal artery blood flow and baseline amino acid concentrations); (2) arginine infusion group (receiving double the arginine amount based on optimal in vitro conditions, 37.66 g/d equivalent to 12.10 g N/d); and (3) alanine iso-nitrogen infusion group (equal nitrogen to arginine group, 77.24 g/d equivalent to 12.13 g N/d). A 3$×$3 Latin square design was employed, with each period lasting 22 days (7-day infusion + 15-day interval). Infusion was administered continuously for 8 hours daily through the jugular catheter, with total infusion volume of 4 L. Infusion solutions were prepared by Nanjing Cambridge Biotechnology Co., Ltd.

### 1.2 Milk Sample Collection and Analysis

Milk samples were collected during the infusion period by recording milk yield at each milking (07:00, 15:00, and 23:00) and collecting one tube per milking. The three daily samples were thoroughly mixed for analysis. Milk composition (fat percentage, non-fat milk solids, protein percentage, and density) was analyzed using a Danish Foss 120 infrared milk analyzer (Yangda Kangyuan Dairy). Casein contents ($\alpha$-casein, $\beta$-casein, and  -casein) were determined using ELISA kits from R&D Systems (CK-E94191B, CK-E94192B, CK-E94193B) according to manufacturer protocols.

### 1.3 Mammary Tissue Sampling and Processing

At the end of each infusion period, approximately 200 mg of mammary tissue was collected from each cow using a biopsy gun and immediately stored in liquid nitrogen (-70°C) for subsequent analysis. Samples were collected from similar locations in alternating mammary quarters across periods. Total RNA was extracted from frozen mammary tissue using the Trizol method. RNA purity and concentration were assessed using 2.2% formaldehyde denaturing gel electrophoresis and an ND1000 microspectrophotometer, with samples stored at -70°C.

### 1.4 Casein Gene Expression Analysis

Complementary DNA (cDNA) was synthesized from total RNA using a TaKaRa reverse transcription kit (reaction prepared on ice). Real-time PCR primers were designed based on bovine mammary casein gene sequences published in GenBank: $\alpha$s1-casein gene (CSN1S1), $\alpha$s2-casein gene (CSN1S2), $\beta$-casein gene (CSN2), and  -casein gene (CSN3). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal reference gene for detecting relative mRNA expression levels. All primers were designed across intron-exon boundaries to avoid genomic DNA contamination and synthesized by Sangon Biotech (Shanghai). Primer sequences are listed in Table 2 . Relative gene expression was quantified using SYBR Green reagent on a 7500 Real-Time PCR System.

Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method with the following formula:

$$\Delta\Delta C_t = (C_{t,\text{target gene in treatment}} - C_{t,\text{reference gene in treatment}}) - (C_{t,\text{target gene in control}} - C_{t,\text{reference gene in control}})$$

$$\text{Gene expression level} = 2^{-\Delta\Delta C_t}$$

Where Ct represents the threshold cycle, defined as the cycle number at which the amplification curve intersects the threshold line.

### 1.5 Data Processing and Statistical Analysis

Data were organized using Excel and analyzed using SPSS 16.0 software for ANOVA and Duncan' s multiple comparison tests. Significance was declared at $P<0.05$.

### 2.1 Effects of Arginine Infusion on Milk Quality

As shown in Table 3 , the arginine infusion group exhibited significantly higher milk protein percentage than both the casein model and alanine iso-nitrogen groups ($P<0.05$). Milk fat percentage was lowest in the casein model group, significantly lower than the other two groups ($P<0.05$). Non-fat milk solids content and milk density did not differ significantly among groups ($P>0.05$). However, both milk yield and milk protein yield were significantly higher in the arginine and alanine iso-nitrogen groups compared to the casein model group ($P<0.05$).

Dynamic changes in milk composition during the infusion period are illustrated in Figures 1 [Figure 1: see original paper], 2 [Figure 2: see original paper], and 3 [Figure 3: see original paper]. Milk protein percentage fluctuated across treatments, with the arginine group maintaining relatively higher levels throughout the infusion period. On day 5, the arginine group was significantly higher than the casein model group ($P<0.05$), while the alanine iso-nitrogen group did not differ significantly from either group ($P>0.05$). Milk fat percentage showed slight fluctuations, with the arginine group significantly exceeding the alanine iso-nitrogen group on day 6 ($P<0.05$) but not differing from the casein model group ($P>0.05$). Non-fat milk solids content also fluctuated, with the arginine group maintaining higher levels and significantly surpassing the casein model group on day 5 ($P<0.05$).

### 2.2 Effects of Arginine Infusion on Casein Content

Table 4 shows that $\alpha$-casein content was lowest in the casein model group, significantly lower than the other two groups ($P<0.05$). $\beta$-casein content did not differ significantly among groups ($P>0.05$). -casein content was highest in the arginine group, significantly exceeding the other groups ($P<0.05$), followed by the alanine iso-nitrogen group, with the casein model group being lowest. Total casein (sum of measured caseins) content and daily yield were highest in the arginine group ($P<0.05$). The proportion of total casein in milk protein ranged from 73.18% to 92.63%, with the arginine group significantly higher than the casein model group ($P<0.05$) but not differing from the alanine iso-nitrogen group ($P>0.05$).

Dynamic changes in individual casein contents are presented in Figures 4 [Figure 4: see original paper], 5 [Figure 5: see original paper], and 6 [Figure 6: see original paper]. $\alpha$-Casein content fluctuated across groups, with the arginine group rising to and maintaining higher levels after infusion initiation. Significant differences among groups emerged from days 3-7 ($P<0.05$), with the arginine group consistently highest. $\beta$-Casein content also fluctuated, with significant differences observed on days 3–4 ($P<0.05$), where the arginine and casein model groups were relatively higher than the alanine iso-nitrogen group. -Casein content remained at higher levels in the arginine group throughout the infusion period, moderate in the alanine iso-nitrogen group, and lowest in the casein model group, with significant differences among groups from days 3–7 ($P<0.05$).

### 2.3 Effects of Arginine Infusion on Casein Gene Expression

Table 5 reveals that casein genes CSN1S1 and CSN1S2 were expressed at significantly lower levels in both the casein model and alanine iso-nitrogen groups compared to the arginine group ($P<0.05$). Expression levels of CSN2 and CSN3 did not differ significantly among the three groups ($P>0.05$).

### 3.1 Effects of Arginine Infusion on Milk Protein Synthesis

Milk composition is generally stable, though individual components such as milk protein fluctuate within certain ranges. Milk protein percentage typically varies between 3.0% and 3.7%, comprising primarily casein, whey proteins, and fat globule proteins. Over 90% of milk protein is synthesized de novo in mammary epithelial cells from amino acids. Studies using 14C-labeled amino acids and 13C-labeled peptides have demonstrated that casein, $\alpha$-lactalbumin, and $\beta$-lactoglobulin in milk are all synthesized from blood-derived amino acids or peptides by mammary epithelial cells. Consequently, the supply and balanced composition of essential amino acids as precursors for milk protein synthesis are critically important.

Recent research further indicates that certain functional amino acids participate in regulating milk protein synthesis through genetic and metabolic pathways beyond serving as substrates. Arginine, as a conditionally essential and functional amino acid, not only contributes to protein synthesis and deposition but also participates extensively in metabolism through various enzymes and metabolites, exerting important metabolic regulatory effects. This study administered arginine infusion based on previous in vitro findings within a casein model framework. The results demonstrated that arginine infusion significantly increased milk yield and milk protein percentage compared to the control group. This effect likely occurred through arginine's regulation of relevant metabolic pathways to enhance mammary lactation performance and protein synthesis.

Arginine can be catabolized to urea and guanylate, subsequently synthesizing polyamines that regulate cell growth. Research on mammary tissue development indicates that arginine is essential for mammary gland development, and its deficiency may reduce mammary DNA and RNA content in rats. Arginine promotes mammary ductal tree development in rats, alveolar development, and proliferation of bovine mammary epithelial cells, providing a foundation for lactation metabolism. Additionally, decreased milk yield is associated with reduced total RNA in mammary epithelial cells, while arginine regulates casein gene mRNA transcription through the Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) and mTOR signaling pathways. Furthermore, arginine stimulates the release of various endocrine hormones including insulin, growth hormone, and prolactin, which may directly or indirectly affect mammary development, lactation performance, and milk protein synthesis, though this requires further investigation.

### 3.2 Effects of Arginine Infusion on Bovine Casein Content

Bovine casein genes span 200 kb on chromosome 6 (6/BTA 6q31-33), forming a gene cluster in the order CSN1S1, CSN1S2, CSN2, and CSN3, encoding $\alpha$s1-casein, $\alpha$s2-casein, $\beta$-casein, and -casein, respectively. $\alpha$s1-Casein and $\beta$-casein are the predominant casein types in milk, while -casein, though present at lower concentrations, remains an important component. In this study, $\alpha$-casein, $\beta$-casein, and -casein contents ranged from 52.70%-62.96%, 27.96%-38.56%, and 9.01%-10.24%, respectively, consistent with typical bovine casein distribution patterns. These results align partially with studies on different cattle breeds showing $\alpha$s1-casein, $\alpha$s2-casein, $\beta$-casein, and -casein contents ranging from 30.74%-32.31%, 5.97%-6.99%, 47.21%-47.63%, and 13.55%-15.28%, respectively.

Arginine significantly increased $\alpha$-casein content and CSN1S1 and CSN1S2 gene expression, consistent with previous findings that arginine enhances $\alpha$s1-casein synthesis and CSN1S1 expression in bovine mammary epithelial cells. Although this study did not separately quantify $\alpha$s1-casein and $\alpha$s2-casein proteins, the gene expression results indicate that arginine upregulated both casein genes in mammary tissue, potentially through signaling pathway regulation and possibly epigenetic modifications, though the latter requires further investigation. Arginine did not significantly increase $\beta$-casein content in this study, contrasting with in vitro results, possibly due to differences between in vivo and in vitro methodologies and inter-tissue metabolic effects. However, arginine infusion significantly increased -casein content, consistent with its effects on -casein content and CSN3 expression in mammary epithelial cells, though the lack of significant CSN3 expression changes warrants further investigation. These results demonstrate that arginine increases $\alpha$-casein and -casein contents, thereby enhancing casein synthesis. The discrepancy with Hu et al.’ s finding that arginine only increased $\beta$-casein in rats likely reflects species differences in casein composition and regulatory responses.

Casein composition, content, and gene polymorphisms are closely associated with lactation performance, milk composition, and dairy processing properties. For instance, -casein glycosylation degree may affect milk and casein yields. As a crucial milk component, CSN3 knockout in rats impairs lactation and destabilizes casein micelles, and its polymorphism correlates with clotting properties and cheese quality. In this study, arginine infusion simultaneously increased both -casein content and CSN3 expression. This suggests that milk protein performance may be altered by changes in -casein synthesis and CSN3 expression, and that increased -casein content may further enhance synthesis of other casein types, though these hypotheses require experimental verification.

In conclusion, arginine infusion increased $\alpha$-casein and -casein contents in milk and upregulated CSN1S1 and CSN1S2 expression, contributing to improved milk protein percentage and quality. These findings provide valuable experimental data for developing milk quality modulation technologies in dairy production.

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