Effects of Jugular Infusion of Arginine on Nutrient Digestion and Nitrogen Utilization in Lactating Dairy Cows (Postprint)

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Abstract

This study primarily investigated the effects of jugular infusion of arginine on nutrient digestion and nitrogen utilization in lactating dairy cows. A 3×3 Latin square experimental design was adopted, with 6 Holstein cows with similar body weight, parity, lactation stage, milk yield, and body condition randomly divided into 3 groups (2 cows per group): casein pattern group (jugular infusion of casein-pattern amino acid mixture, control group), arginine group (jugular infusion of arginine), and alanine group (jugular infusion of alanine isonitrogenous with the arginine group); each experimental period lasted 22 days, including a 7-day infusion period and a 15-day interval period. Urea nitrogen concentrations in urine, serum, and milk, as well as indices of nutrient apparent digestibility were measured. The results showed that urea nitrogen concentrations in urine and serum did not differ significantly among groups ($P>0.05$), while milk urea nitrogen concentration in the arginine group was significantly lower than that in the control and alanine groups ($P<0.05$); the apparent digestibility of dry matter, organic matter, crude protein, and neutral detergent fiber in the arginine group were all significantly higher than those in the alanine group ($P<0.05$); milk protein nitrogen yield was highest in the arginine group, significantly higher than the other two groups ($P<0.05$); and the proportion of milk protein nitrogen to nitrogen intake was also highest in the arginine group. In conclusion, arginine infusion has a certain promoting effect on nitrogen digestion and utilization in mid-lactation dairy cows, and can increase milk protein nitrogen yield and its proportion to nitrogen intake.

Full Text

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Abstract: This study investigated the effects of jugular vein infusion of arginine on nutrient digestion and nitrogen utilization in lactating dairy cows. Six Holstein cows with similar body weight, parity, lactation stage, milk yield, and body condition were randomly allocated to three groups (2 cows per group) in a 3×3 Latin square design: casein pattern group (infusion of casein-pattern amino acid mixture through jugular vein, control group), arginine group (arginine infusion through jugular vein), and alanine group (alanine infusion through jugular vein, iso-nitrogenous with arginine group). Each experimental period lasted 22 days, comprising a 7-day infusion period and a 15-day interval period. Urea nitrogen concentrations in urine, serum, and milk, as well as nutrient apparent digestibility indices, were measured. The results showed no significant differences in serum and urine urea nitrogen concentrations among groups ($P>0.05$). However, milk urea nitrogen concentration in the arginine group was significantly lower than that in the control and alanine groups ($P<0.05$). The apparent digestibility of dry matter, organic matter, crude protein, and neutral detergent fiber in the arginine group were all significantly higher than those in the alanine group ($P<0.05$). Milk protein nitrogen yield was highest in the arginine group, significantly exceeding the other two groups ($P<0.05$). The ratio of milk protein nitrogen to nitrogen intake was also highest in the arginine group. In conclusion, arginine infusion promotes nitrogen digestion and utilization in mid-lactation dairy cows, thereby increasing milk protein nitrogen yield and its proportion of total nitrogen intake.

Keywords: arginine infusion; nitrogen utilization; nutrient digestion; dairy cow

Urea nitrogen recycling in ruminants plays a crucial role in maintaining nitrogen balance [1]. Approximately 40–80% of urea synthesized in the liver enters the digestive tract [2]. Previous research reported that 33% of urea produced in ruminant liver is excreted via urine, while 67% enters the digestive tract, with 10% of the latter subsequently excreted in feces [3]. Nitrogen excreted through feces and urine indicates incomplete nitrogen utilization and poses an environmental threat that intensifies with increasing livestock production scale. Therefore, improving nitrogen utilization efficiency and reducing nitrogen excretion in ruminants has become increasingly urgent. Arginine (Arg) is one of the most functionally diverse amino acids and carries the highest nitrogen content among the 20 basic amino acids constituting biological proteins. In mammals, ornithine in the urea cycle can be converted to Arg, and conversely, Arg can

be converted to urea and ornithine via arginase catalysis, thereby participating in nitrogen metabolism [4]. Dietary Arg supplementation promotes nitrogen deposition and improves daily weight gain and feed conversion ratio in pigs [5]. Rats fed Arg-deficient diets exhibit weight loss and negative nitrogen balance [6]. Additionally, the Arg-nitric oxide (NO) pathway promotes mammary gland and intramammary vascular growth, enhances nutrient uptake, and improves mammary tissue development, thereby increasing lactation performance [7]. As early as 1982, Pau et al. [8] demonstrated that feeding Arg-deficient diets to pregnant rats reduced mammary DNA and RNA content, concluding that Arg is essential for normal mammary differentiation. Alkareem et al. [9] treated pregnant rats with Arg and NO inhibitors, finding that Arg promoted mammary ductal tree and alveolar development. Our previous research using bovine mammary epithelial cells yielded similar results, showing that Arg promotes cell proliferation and casein synthesis, and elucidated that Arg enhances casein expression through the mTOR pathway [10]. However, whether Arg simultaneously promotes nitrogen digestion and absorption metabolism while enhancing mammary casein synthesis remains unclear. This experiment altered Arg supply in mid-lactation cows through jugular infusion of different amino acids to observe changes in lactation performance, nitrogen balance, and nutrient digestion, thereby investigating whether Arg affects lactation performance by improving nitrogen utilization.

1.1 Experimental Design

Six Holstein cows from the experimental farm of Yangzhou University were selected based on similar body weight, parity (4th parity), lactation stage [(80±2) days], milk yield [(21.0±1.0) kg/d], and body condition score (3.0). The basal diet was formulated according to NRC (2001) dairy cattle feeding standards. Diet composition and nutrient levels are presented in Table 1 , where Chinese wildrye was fed separately and other ingredients were provided as total mixed ration. Cows were housed individually with consistent management, fed equal amounts twice daily (Chinese wildrye first, followed by mixed feed), and milked three times daily.

A 3×3 Latin square design was adopted, with cows randomly divided into three groups: casein pattern group (infusion of casein-pattern amino acid mixture through jugular vein, control group), Arg group [based on in vitro cell experiment results, the optimal Arg concentration for promoting casein expression was selected at 2× concentration, infusion rate of 37.66 g/(L · d)], and alanine group [iso-nitrogenous with Arg group, infusion rate of 77.24 g/(L · d)]. Each period lasted 22 days, including a 7-day infusion period and a 15-day interval period. Amino acid infusions were prepared by Nanjing Cambridge Biotechnology Co., Ltd. and infused continuously for 8 hours daily through indwelling jugular needles, with a total infusion volume of 4 L per cow per day.

Table 1 Composition and nutrient levels of basal diets (DM basis) %

Ingredients	Content	Nutrient levels[2])	Content
Alfalfa		NEL/(MJ/kg)	
Chinese wildrye		CP	
Corn silage		NFC	
Corn		NDF	
Cottonseed meal		ADF	
Soybean meal		EE	
DDGS		Ca	
CaHPO			
NaCl			
Premix[1])			
Total			

[1]) The premix provided per kg of diet: CuSO 25 mg, FeSO ·H O 75 mg, ZnSO · H O 105 mg, Co 0.0024 mg, Na SeO 0.016 mg, VA 12,000 IU, VD 10,000 IU, VE 25 mg, Nicotinic acid 36 mg, Choline 1,000 mg.
[2]) NEL was a calculated value, while other nutrient levels were measured values.

1.3 Measurements and Calculations

During the 15-day preliminary period, cows were adapted to the basal diet and observed for feed intake. During the 7-day infusion period, daily feed intake and orts were weighed, dry matter content was determined, and average daily dry matter intake (DMI) was calculated. On the last two days of infusion, blood samples were collected from the coccygeal artery before morning feeding into coagulation tubes, then centrifuged at 4°C and 447.2×g for 20 minutes to obtain serum for urea nitrogen determination.

Urine was collected as completely as possible during the last two days of infusion, with 2 mL of 2% sulfuric acid and 4 drops of toluene added per 100 mL urine. Staff collected urine promptly when cows urinated into deep buckets containing pre-added sulfuric acid based on estimated urine volume from the preliminary period. Each collection was weighed, and 100 mL samples were taken and mixed for urea nitrogen analysis.

Milk yield was recorded during morning, midday, and evening milking on the last two days of infusion, and average yield was calculated. Milk samples were collected at each time point, mixed thoroughly, and used for urea nitrogen determination. Milk protein nitrogen yield was measured using a Foss 120 infrared milk analyzer (Foss, Denmark). Urea nitrogen concentrations in serum, milk, and urine were determined using a urea nitrogen test kit (C013-1, Nanjing Jiancheng Bioengineering Institute) following the manufacturer' s instructions.

Fecal samples were partially collected during the last two days of infusion, with 20 mL of 2% sulfuric acid added per 100 g feces for nitrogen fixation. Feces were dried at 65°C, ground, and analyzed for organic matter, crude protein,

neutral detergent fiber (NDF), and acid detergent fiber (ADF) content using methods described by Zhang Liying [11]. Daily diet samples were collected in equal amounts, mixed thoroughly, and analyzed for the same components using the same methods [11].

Apparent digestibility was calculated using acid-insoluble ash (AIA) as an internal marker [12]:
Nutrient apparent digestibility (%) = 1 -(b×c)/(a×d)
where a = nutrient content in diet, b = nutrient content in feces, c = AIA content in diet, and d = AIA content in feces.

Nitrogen utilization indices were calculated as follows:
- Fecal nitrogen/DMI (%) = fecal nitrogen/dry matter intake
- Milk protein nitrogen/N intake (%) = milk protein nitrogen/nitrogen intake
- Metabolic nitrogen/N intake (%) = (N intake -fecal N -urinary N)/N intake
- Nitrogen balance = N intake -fecal N -urinary N -milk protein N

1.4 Statistical Analysis

Data were processed using Excel 2013 and analyzed using SPSS 16.0 software for ANOVA and Duncan' s multiple comparison tests. Significance was declared at $P<0.05$.

2.1 Urea Nitrogen Concentrations in Urine, Serum, and Milk

As shown in Figure 1 [Figure 1: see original paper], no significant differences were observed in serum and urine urea nitrogen concentrations among groups ($P>0.05$), though serum urea nitrogen in the Arg group was numerically lower than in the casein pattern and alanine groups. Milk urea nitrogen concentration in the Arg group was significantly lower than in the casein pattern and alanine groups ($P<0.05$), while no significant difference was found between the latter two groups ($P>0.05$).

2.2 Nutrient Apparent Digestibility

Figure 2 [Figure 2: see original paper] shows that dry matter intake did not differ significantly among groups ($P>0.05$). However, apparent digestibility of dry matter and organic matter differed significantly ($P<0.05$), with the casein pattern and Arg groups showing similar values that were both significantly higher than the alanine group ($P<0.05$).

As illustrated in Figure 3 [Figure 3: see original paper], no significant difference was observed in apparent digestibility of crude protein and neutral detergent fiber between the casein pattern and Arg groups ($P>0.05$), but the Arg group was significantly higher than the alanine group ($P<0.05$). Acid detergent fiber apparent digestibility did not differ significantly among the three groups ($P>0.05$), though the Arg group showed numerically higher values than the other groups.

2.3 Nitrogen Balance

Table 2 presents nitrogen utilization data. No significant differences were found in nitrogen intake and fecal nitrogen excretion among groups (P>0.05). The fecal nitrogen/DMI ratio in the Arg group was significantly lower than in the alanine group (P<0.05) but did not differ from the casein pattern group (P>0.05). Urinary nitrogen excretion differed significantly among groups (P<0.05), with the alanine group being significantly higher than the casein pattern group (P<0.05), which in turn was significantly higher than the Arg group (P<0.05). Milk protein nitrogen yield differed significantly among groups (P<0.05), being highest in the Arg group, followed by the alanine group, and lowest in the casein pattern group. The ratio of milk protein nitrogen to nitrogen intake was also highest in the Arg group, followed by the alanine and casein pattern groups, though differences were not statistically significant (P>0.05). The ratio of metabolic nitrogen to nitrogen intake ranked as Arg group > casein pattern group > alanine group. Nitrogen balance values were 121.19, 108.50, and 83.65 g/d for the casein pattern, Arg, and alanine groups, respectively, with the alanine group showing the lowest value.

Table 2 Effects of Arg infusion on nitrogen utilization of lactating cows

Items	Casein pattern group	Arg group	Alanine group	P-value
N intake (g/d)				<0.01
Feces nitrogen (g/d)				<0.01
Feces-N/DMI (%)	0.76ab	0.73b	0.79a	<0.01
Urine N (g/d)	111.36b	93.47c	124.94a	<0.01
Milk yield (kg/d)	21.45b	23.65a	23.65a	<0.01
Milk protein N (g/d)	103.32c	115.21b	125.78a	<0.01
Milk protein N/N intake (%)	0.73b	0.76ab	0.79a	<0.01
Metabolism N/N intake (%)				
Nitrogen balance (g/d)	121.19	108.50	83.65	

Values in the same row with different letter superscripts differ significantly (P<0.05).

3.1 Effects of Arginine Infusion on Milk Urea Nitrogen Concentration and Milk Protein Nitrogen Yield

Rumen microorganisms can hydrolyze urea to CO and ammonia via urease, which then aminates keto acids to form amino acids for microbial protein (MCP) synthesis. MCP is subsequently degraded by small intestinal proteases into various amino acids for absorption and utilization [13]. Studies using ^{1} N-labeled urea [14] and ^{1} N-labeled ammonium salts [15] demonstrated that labeled nitrogen can be transferred to amino acids for body and milk protein synthesis. As urea is the primary end product of ammonia and amino acid metabolism

in ruminants [13], serum urea nitrogen concentration accurately reflects amino acid and protein metabolism status [16]. Since urea nitrogen diffuses freely and rapidly across cell membranes, milk urea nitrogen has been used as a substitute for serum urea nitrogen to monitor dietary protein levels and predict environmental nitrogen excretion. Li et al. [17] reported that milk urea nitrogen concentration can monitor dietary protein metabolism, while Zou et al. [18] further demonstrated a negative linear correlation between milk urea nitrogen and milk protein percentage, making it an indicator of nitrogen utilization efficiency and protein nutritional status. Our results showed that Arg infusion significantly reduced milk urea nitrogen concentration while numerically decreasing serum urea nitrogen. Concurrently, Arg infusion increased milk protein nitrogen yield and its proportion of nitrogen intake, suggesting that Arg may enhance milk protein synthesis by improving nitrogen utilization efficiency.

3.2 Effects of Arginine Infusion on Nitrogen Utilization

Ruminant nitrogen metabolism encompasses dietary nitrogen intake, protein digestion and degradation in the rumen and lower digestive tract, protein absorption in the gastrointestinal tract, and amino acid utilization by peripheral tissues [19]. Fecal and urinary nitrogen represent two loss pathways of ingested nitrogen. Fecal nitrogen comprises undigested nitrogen passing through the digestive tract, largely influenced by dietary protein level, whereas urinary nitrogen consists of deaminated amino acids not utilized for tissue protein synthesis, primarily affected by dietary amino acid balance [20]. Therefore, both dietary crude protein level and amino acid balance influence nitrogen utilization. Studies show that increasing dietary protein level raises nitrogen intake and tends to increase fecal nitrogen excretion, with nitrogen deposition decreasing significantly when dietary crude protein exceeds 11.0% [21]. Optimal nitrogen deposition and amino acid utilization are achieved when non-essential amino acids provide 30–45% of daily nitrogen consumption in rats [22]. In our experiment, the alanine group showed significantly lower crude protein apparent digestibility than the Arg group and numerically lower than the casein pattern group, while urinary nitrogen excretion was significantly higher than in the other two groups. This likely resulted from increased crude protein content and amino acid imbalance caused by additional alanine infusion on top of the casein pattern baseline.

When dairy cows are fed to meet requirements under stable conditions, fecal nitrogen excretion maintains a relatively stable ratio to DMI of approximately 0.60% [23]. In this trial, fecal nitrogen/DMI ratios ranged from 0.73% to 0.79%, slightly higher than 0.60%, possibly because nitrogen supply exceeded the requirement for a 550 kg Holstein cow producing 21 kg/d milk (4% fat), whose metabolic protein requirement is 1,720.98 g/d (equivalent to 275.34 g N/d, with estimated nitrogen intake of approximately 447.71 g/d) [24]. Among groups, the Arg group had a significantly lower fecal nitrogen/DMI ratio than the alanine group. With no significant difference in total nitrogen supply (464.59 g/d vs. 477.01 g/d), the reduced fecal nitrogen/DMI ratio primarily indicates im-

proved nitrogen digestion and utilization efficiency. This is corroborated by lower milk urea nitrogen concentration and urinary nitrogen excretion in the Arg group, collectively demonstrating that Arg infusion enhances nitrogen digestion and absorption to increase milk protein synthesis. Compared with the casein pattern group, the Arg group showed no significant differences in crude protein apparent digestibility or fecal nitrogen/DMI ratio, yet achieved higher milk protein nitrogen yield and ratio to nitrogen intake, with slightly lower total nitrogen intake numerically. This may be attributed to Arg' s ability to promote mammary tissue development [9], alveolar growth, and casein expression [25], thereby enhancing milk protein synthesis.

Notably, all groups exhibited relatively high nitrogen balance values, likely because: (1) milk protein was measured using a FOSS MilkoScan FT120 infrared analyzer detecting peptide bonds (protein nitrogen) rather than Kjeldahl crude protein nitrogen, with some nitrogen loss from milk urea nitrogen; (2) the experimental cows were 4th parity with milk yield of only ~21 kg/d at 80 days in milk, possibly trending toward body weight gain; and (3) feeding levels may have exceeded nutritional requirements, causing weight gain and reduced milk production. Zhang et al. [26] reported relatively high nitrogen balance values of 215.76, 144.24, and 100.58 g/d for early-, mid-, and late-lactation cows, respectively. Specific reasons require further verification.

3.3 Effects of Arginine Infusion on Neutral Detergent Fiber Apparent Digestibility

Arginine infusion also improved dietary neutral detergent fiber apparent digestibility. As structural carbohydrates, NDF is degraded in the rumen into volatile fatty acids (VFA) and keto acids, providing energy for rumen microorganisms and host production [27]. Approximately 75% of rumen-generated VFA is absorbed by ruminal epithelial cells, with the remainder absorbed in the lower digestive tract [28]. Ruminants can also absorb VFA from the cecum. Warner et al. [29] and Davis [30] reported maximum post-ruminal cellulose digestion of 44.5 g/d in sheep and 143 g/d in steers via abomasal infusion of pure wood pulp cellulose. Arginine promotes intestinal mucosal repair [31] and alleviates intestinal damage caused by allergy and endotoxins [32]. As an NO precursor, Arg enhances NO production to scavenge oxygen free radicals and reduce lipid peroxidation injury to intestinal mucosa [33]. Sukhotnik et al. [34] found that Arg influences small intestinal absorptive function. Collectively, Arg may enhance NDF metabolite absorption by promoting intestinal absorptive function, ultimately improving NDF digestibility. Combined with higher crude protein apparent digestibility and lower milk and serum urea nitrogen concentrations, Arg infusion appears to improve both energy and nitrogen supply [35].

In conclusion, jugular vein infusion of Arg reduced milk urea nitrogen concentration, increased crude protein digestibility, and enhanced milk protein nitrogen yield and its proportion of nitrogen intake, demonstrating that Arg improves milk protein synthesis by enhancing nitrogen digestion and utilization in dairy

cows.

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