Dietary supplementation with N-carbamoylglutamate initiated from the prepartum stage improves lactation performance of postpartum dairy cows

Fengfei Gu, Chao Miao, Luyi Jiang, Diming Wang, Hongyun Liu, Jianxin Liu*

Institute of Dairy Science, MoE Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, 310058, China

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ABSTRACT

The objective of this study was to investigate the effects of supplementing N-carbamoylglutamate (NCG), an Arg enhancer, on amino acid (AA) supply and utilization and productive performance of early-lactating dairy cows. Thirty multiparous Chinese Holstein dairy cows were randomly divided into control (CON, n = 15) and NCG (CON diet supplemented with NCG at 20 g/d per cow, n = 15) groups at 4 wk before calving. Diets were offered individually in tie-stalls, and NCG was supplemented by top-dress feeding onto total mixed ration for the NCG group. The experiment lasted until wk 10 after calving. Dry matter intake tended to be higher (P = 0.06), and yields of milk (P < 0.01), milk protein (P < 0.01), and milk fat (P < 0.01) were higher in the NCG-cows than in the CON-cows. Plasma activities of aspartate aminotransferase (P < 0.01), alanine aminotransferase (P = 0.03), and plasma level of β-hydroxybutyrate (P = 0.04) were lower in the NCG-cows than in the CON-cows. Plasma glucose (P = 0.05) and nitric oxide (NO, P < 0.01) concentrations were greater. Coccygeal vein concentrations of Cys (P < 0.01), Pro (P < 0.01), Tyr (P = 0.05), most essential AA except Thr and His (P < 0.01), total essential AA (P < 0.01), and total AA (P < 0.01) were higher in the NCG-cows than in the CON-cows. The arterial supply of all AA was greater in the NCG-cows than in the CON-cows. The NCG-cows had higher mammary plasma flow of AA (P = 0.04) and clearance rate of Cys (P < 0.01), Pro (P < 0.01) and Asp (P < 0.01), and higher ratios of uptake to output of Met (P = 0.05), Lys (P < 0.01), Cys (P = 0.01), Pro (P = 0.03), and Asp (P = 0.01). In summary, addition of NCG initiated from the prepartum period improved the lactation performance of postpartum dairy cows, which might attribute to greater Arg and NO concentrations, as well as improved AA supply and utilization, liver function, and feed intake in these cows.

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1. Introduction

During the perinatal period, cows enter a state of negative energy balance and face a series of great physiological changes, including dietary changes, parturition, and lactation onset. All these conditions pose significant challenges to metabolic homeostasis (Sun et al., 2016). Increasing dry matter intake (DMI) of post-calving cows is considered to be an efficient way to meet their energy requirements and improve milking performance (Zhou et al., 2016a, 2016b). Also, adequate amino acid (AA) supplies are essential for both milk production and milk protein synthesis. However, free AA concentration in the plasma of postpartum dairy cows decreases abruptly compared with that in pre-calving period (Meijer et al., 1995), indicating the increased utilization of AA for lactation. Thus, more AA supplies would be needed for milk synthesis in early-lactating cows. Amino acids are also important precursors of hepatic gluconeogenesis in dairy cows. Addition of gluconeogenic substrates during the transition period could improve the postpartum lactation performance (White, 2015). Improving the availability of limiting AA (Met and Lys) increased DMI and milk
production of dairy cows during early lactation (Osorio et al., 2013; Batistel et al., 2017). However, information is limited for other essential amino acids (EAA).

Arg is an EAA for dairy cows (NRC, 2001) and is considered to be a functional AA with many biological functions (Wu et al., 2009). For example, nitric oxide (NO), an intermediate of Arg metabolism, can regulate angiogenesis and molecular signaling transfer in various cells (Jobgen et al., 2006). The roles of Arg are recognized in reproduction, immunity, neonatal growth, and wound healing in monogastric animals (Wu et al., 2009). However, limited studies have been conducted on the role of Arg in milk protein synthesis in lactating ruminants (Doepel and Lapierre, 2011; Ding et al., 2018), possibly due to the degradation of unprotected Arg in the rumen and the high price of rumen-protected Arg (Chacher et al., 2012). Alternatively, N-carbamoylglutamate (NCG) is a structural analog of N-acetylglutamate, which is essential in endogenous Arg synthesis (Gessler et al., 2010). Therefore, as an Arg enhancer in ruminants, NCG has unique advantages, such as low degradability in the rumen, reduced cost, and lack of negative effects on intestinal absorption of dietary Trp, His, and Lys (Chacher et al., 2012). NCG is a potential alternative for dairy cows.

The rapid decline in plasma Arg concentrations before parturition (Meijer et al., 1995) indicates a high Arg requirement for dairy cows at this stage. Therefore, the objective of the study was to determine the effects of dietary NCG on AA supply, DMI, milk production, and liver function of dairy cows, and to provide a nutritional strategy for the healthy feeding during transition period.

2. Materials and methods

2.1. Animals and experimental design

The experiment was approved by the Animal Care Committee of Zhejiang University and conducted at Hangjiang Dairy Farm (Hangzhou, China). Thirty multiparous Chinese Holstein dairy cows were selected at 4 wk before the expected calving date and divided to 15 blocks based on body condition scores (BCS; 3.39, SD = 0.51), body weight (BW; 657 kg, SD = 58), parity (2.73, SD = 0.98) and 305-d milk yield (8,692 kg, SD = 607) of the previous lactation. Body weight was estimated based on the method described by Yan et al. (2009). Body condition was scored following the method described by Edmonson et al. (1989), using a 5-point scale (1 = thin, 5 = fat) at 3 time points (06:00, 14:00, 20:00). The animals in each block were randomly allocated into 2 treatments, basal diets without (control, CON) or with NCG (Beijing Animore Sci. & Tech. Co., Ltd., Beijing, China) at 20 g/d. The experiment lasted until 10 wk after calving. Throughout the whole experimental process, cows were housed in a barn with individual tie-stalls and had free access to water. NCG was added once per day at 14:00 by scattering it on the feed and the high price of rumen-protected Arg (Chacher et al., 2012). Therefore, as an Arg enhancer in ruminants, NCG is a potential alternative for dairy cows.

2.2. Sample collection and analysis

Dry matter intake was recorded for 2 consecutive days at 2 wk before calving (7 to 9 d before the expected calving date) and every other week after calving. Samples of total mixed ration andorts were collected on the same days and analyzed for dry matter (105 °C for 5 h), crude protein (method 988.05; AOAC, 1990), acid detergent fiber (ADF) (method 973.18; AOAC, 1990) and neutral detergent fiber (NDF) (Van Soest et al., 1991). Ingredients and chemical composition of the basal diet are listed in Table 1.

Milk yield was recorded weekly for 2 consecutive days during the post-calving stage, and a milk sample was collected from individual cows following a ratio of 4:3:3 (morning, afternoon, and evening). Milk samples were mixed with bronopol (milko preservative; D & F Control Systems Inc., San Ramon, CA, USA) for analysis of milk composition (fat, protein, lactose, milk urea nitrogen [MUN], total solid [TS], and somatic cell counts [SCC]) through an infrared analysis system using a 4-channel spectrophotometer (MilkoScan; Foss Electric A/S, Hillerod, Denmark).

Blood samples were collected from the coccygeal vein of each cow and deposited into 10-ml tubes containing an anticoagulant (heparin lithium) 3 h after morning feeding at wk 3, 10 wk postpartum, 3, 7, and 10 relative to calving. Another copy of plasma was sampled from the coccygeal artery and superficial epigastric vein at the end of wk 3 and 10 at 07:00, 14:00, and 20:00 postpartum with 10-ml tubes containing an anticoagulant (heparin lithium). Blood samples at wk 3 were not collected for 2 cows in the CON group and 3 cows in the NCG group due to earlier (2 to 3 d earlier) than expected parturition. The samples were centrifuged at 3,000 × g for 15 min to collect plasma which was then frozen at −20 °C until subsequent analysis. Plasma biochemical variables were analyzed using an AutoAnalyzer 7020.

Table 1 Ingredients and chemical composition of the basal diet (% DM basis).

| Item                     | Dry period | Lactation period |
|--------------------------|------------|------------------|
| Ingredients              |            |                  |
| Alfalfa                  | 14.1       | 14.1             |
| Corn silage              | 19.8       | 19.8             |
| Corn grain, ground       | 15.4       | 15.2             |
| Soybean meal             | 10.3       | 10.3             |
| Steam-flaked corn        | 12.7       | 12.7             |
| Sugar beet pulp          | 6.97       | 6.97             |
| Dicalcium phosphate      | 0.12       | 0.12             |
| Sodium bicarbonate       | 0.50       | 0.50             |
| Limestone, ground        | 0.76       | 0.76             |
| Fatty acid calcium       | 1.50       | 1.50             |
| Mycotoxin binder         | 0.10       | 0.10             |
| Wheat bran               | 6.85       | 6.85             |
| Rice straw               | 17.9       | 17.9             |
| Beer grains              | 1.43       | 1.43             |
| Oat hay                  | 7.05       | 7.05             |
| Yeast                    | 0.10       | 0.10             |
| Salt                     | 0.51       | 0.51             |
| Premix 1 (dry period)    | 0.86       | 0.86             |
| Premix 2 (lactation period) | 0.56    | 0.56             |
| Chemical composition     |            |                  |
| Crude protein            | 12.6       | 17.3             |
| Neutral detergent fiber  | 40.0       | 32.6             |
| Acid detergent fiber     | 22.9       | 19.4             |
| Ca                      | 0.60       | 1.10             |
| P                       | 0.40       | 0.41             |
| Crude ash                | 6.85       | 6.83             |
| NEI                      | 5.77       | 7.03             |

1 Formulated to provide for one kilogram of premix: 220,000 to 400,000 IU vitamin A; ≥ 2,250 IU vitamin E; 50,000 to 100,000 IU vitamin D2; ≥ 380 mg niconitramine; 0.2 to 0.7 g Cu; 1.0 to 2.4 g Zn; 0.8 to 3.0 g Mn; 12.5 to 100 mg I; ≥ 8 to 25 mg Se; 0.05 to 0.15 kg NaCl; 0.05 to 0.15 kg Ca; and ≥ 0.015 kg total P.

2 Formulated to provide for one kilogram of premix: 960,000 to 1,440,000 IU vitamin A; ≥ 240,000 to 360,000 IU vitamin D2; 7,000 IU vitamin E; 1,000 mg niconitramine; 200 mg biotin; 32 to 48 mg Se-yeast; 6,400 to 9,600 mg Zn; 64 to 96 mg Se; 144 to 216 mg I; 2,500 to 3,600 mg Fe; ≥ 48 to 72 mg Co; ≥ 5,600 to 8,400 mg Mn; 2,000 to 3,000 mg Cu and ≤ 0.10 kg water.

3 Net energy for lactation, calculated based on the Ministry of Agriculture of China recommendations (MOA, 2004).
instrument (Hitachi High-technologies Corporation, Tokyo, Japan) with colorimetric commercial kits (Ningbo Medical System Biotechnology Co., Ltd.) to determine urea nitrogen, total protein, albumin, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), triglyceride, glucose, total bilirubin, cholesterol, globulin, glutamic oxaloacetic transaminase (AST), and glutamic-pyruvic transaminase (ALT) (Gu et al., 2018). Plasma concentration of NO was measured using the nitrate reduce method with a NO assay kit (Nanjing Jiangcheng Bioengineering Research Institute, Nanjing, China, No. A012-1-2). Total NO synthase was measured with a commercial test kit (Nanjing Jiangcheng Bioengineering Research Institute, Nanjing, China. No. A014-2-1).

Amino acid profiles in plasma from the coccygeal vein were determined at wk 3 and 10 relative to parturition with an Automatic AA Analyzer (Hitachi High-technologies Corporation, Tokyo, Japan) as previously described (Wang et al., 2016). Also, AA profiles of milk and plasma from the coccygeal artery and superficial epigastric vein were determined at the end of wk 3 and 10 postpartum with the same method.

2.3. Calculations

The indexes related to AA utilization by the mammary gland were calculated (Cant et al., 1993) as below.

Mammary plasma flow (MPF, L/d) = [Milk (Phe + Tyr) (µmol/d) x 0.965]/Arterial and venous (AV) difference of (Phe + Tyr) (µmol/L)

Arterial free AA supply (µmol/d) = Arterial free AA concentration (µmol/L) x MPF (L/d)

Clearance rate of AA in the mammary gland was calculated using the following model of Hanigan et al. (1998).

Clearance rate (L/h) = MPF (L/h) x AV difference of AA (µmol/L)

Venous concentration of AA (µmol/L)

Uptake-to-output ratio = AA uptake in the mammary gland (µmol/d)/AA output in milk (µmol/d)

Positive values of clearance rates indicate uptake, and negative values indicate output by the mammary gland.

2.4. Statistical analysis

Kolmogorov–Smirnov test was used to evaluate the normal distribution, and Levene test was used to evaluate the homogeneity of variance using SPSS software (version 20.0). As energy-corrected milk yield (ECM) and SCC were not normally distributed, natural logarithm was used to transform these parameters to normal distributions.

All data were analyzed using SAS software (version 9.0) with covariance type AR(1) for repeated measures with mixed models. A randomized complete block design was used for repeated measures in the analysis of treatment × week as fixed effects and the block and cow as a random effect. The effect of week was included as a repeated measure. Cows within diets were subjects for repeated measurements. Means were separated using the PDIF option of LSMEANS statement. The experimental results were reported as least-squares means. Significance was declared at P ≤ 0.05, and 0.05 < P ≤ 0.10 was considered a trend.

3. Results

3.1. Dry matter intake and milk production

The results for DMI and milk production are presented in Table 2 and Fig. 1. Dry matter intake tended to be greater (P = 0.06) in the NCG-cows than in CON-cows. Yields of milk (P < 0.01), protein (P = 0.02), fat (P < 0.01), and ECM (P < 0.01) were greater in the NCG-cows than in the CON-cows. Milk urea nitrogen concentration was lower (P < 0.01) in the NCG-cows than in the CON-cows. Milk lactose (P = 0.06) and total solid (P = 0.10) contents tended to be higher in the NCG-cows than in the CON-cows. No differences were found in contents of protein (P = 0.24) and fat (P = 0.27) between the 2 dietary groups. In addition, significant interaction effect was found for yields of milk (P = 0.08), protein (P < 0.01) and fat (P = 0.07), as well as for contents of protein (P = 0.08), fat (P = 0.06) and MUN (P < 0.01).

3.2. Plasma parameters

Plasma parameters are presented in Table 3, and changes in BHB, NEFA, NO, NO synthase and cholesterol are shown in Fig. 2. Plasma concentrations of BUN (P = 0.01) and BHB (P = 0.05), and activities of AST (P < 0.01) and ALT (P = 0.03) were lower; and triglyceride (P = 0.09) tended to be lower in the NCG-cows than in the CON-cows. In contrast, plasma concentration of NO (P < 0.01) were greater and plasma concentrations of glucose (P = 0.05) and cholesterol (P = 0.10; T x W, P < 0.01), and the activity of NO synthase (T x W; P = 0.06) tended to be greater in the NCG-cows than in the CON-cows. No significant differences were found in plasma concentrations of NEFA (P = 0.44), total protein (P = 0.84), albumin (P = 0.99), or globulin (P = 0.72) between 2 treatments.

3.3. AA profiles in plasma and utilization of AA by the mammary gland

Compared with the CON-cows, the NCG-cows had higher plasma concentrations of EAA other than Thr and His (P < 0.05, Table 4), especially for the concentration of Arg which was increased by 30%. In terms of non-EAA (NEAA), the concentrations

Table 2

| Item                  | CON  | NCG  | SEM  | P-value |
|-----------------------|------|------|------|---------|
|                      | T    | W    | T x W|         |
| Yield, kg/d           | 22.3 | 23.3 | 0.52 | 0.06    | <0.01  | 0.72 |
| Milk                  | 37.7 | 40.9 | 0.89 | <0.01   | <0.01  | 0.08 |
| Protein               | 3.73 | 3.82 | 0.02 | <0.01   | <0.01  | 0.20 |
| Fat                   | 1.13 | 1.20 | 0.02 | <0.01   | <0.01  | 0.01 |
| Lactose               | 1.55 | 1.74 | 0.04 | <0.01   | <0.01  | 0.07 |
| Protein               | 2.95 | 2.88 | 0.05 | <0.01   | <0.01  | 0.08 |
| Fat                   | 4.04 | 4.16 | 0.08 | <0.01   | <0.01  | 0.06 |
| Lactose               | 5.02 | 5.10 | 0.03 | <0.01   | <0.01  | 0.05 |
| Total solid           | 12.4 | 12.6 | 0.10 | <0.01   | <0.01  | 0.34 |
| MUN, mg/N/d           | 12.9 | 11.2 | 0.32 | <0.01   | <0.01  | 0.01 |
| In (SCC), 10^3/mL     | 4.20 | 3.82 | 0.19 | <0.01   | <0.01  | 0.22 |
| Feed efficiency†      | 1.88 | 1.99 | 0.06 | 0.13    | 0.23   | 0.37 |

T = treatment; W = week; T x W = interaction between treatment and week; DMI – dry matter intake; ECM – energy-corrected milk yield; MUN – milk urea nitrogen; SCC – somatic cell count.  
† Feed efficiency = milk yield (kg/d)/DMI (kg/d).
of Cys (P < 0.01), Tyr (P = 0.05) and Pro (P < 0.01) were greater in the NCG-cows than in the CON-cows. The concentrations of total EAA (P < 0.01) and total AA (P < 0.01) were greater in the NCG-cows than in the CON-cows. Amino acid supplies to the mammary gland via arterial were significantly greater in the NCG-cows than those in the CON-cows (P < 0.05). Compared with the CON-cows, the NCG-cows had greater MPF (P = 0.04).

The clearance rate of Cys, Pro, and Asp were higher in the NCG-cows than in the CON-cows (P < 0.01). Uptake-to-output (U:O) ratios of AA across the mammary gland for Met (P = 0.05), Lys (P < 0.01), Cys (P = 0.01), Pro (P = 0.03) and Asp (P = 0.01) were greater in the NCG-cows than in the CON-cows. The clearance rate (P = 0.08) and U:O ratio (P = 0.10) of NEAA tended to be higher in the NCG-cows (Table 5).

4. Discussion

A critical issue for transition cows is that DMI does not meet the needs of rapidly increasing milk production. In the current study, we found that supplementation of NCG initiated from the pre-partum stage increased the AA supply, DMI, and production performance of postpartum cows. This finding may be attributed to the addition of NCG increased production of endogenous Arg and NO and improved liver function.

Dietary supplementation with NCG may increase blood Arg concentrations in gilts (Zhu et al., 2015) and rats (Cao et al., 2016). In the current study, plasma Arg concentrations increased with the addition of NCG. Additionally, other EAA (except Thr and His) and total AA were greater in cows supplemented with NCG. NO is an important signaling molecule produced in Arg metabolic cycle (Yang and Denbow, 2007), which was also found in the results of the current study. Increased blood NO concentration may improve MPF of dairy cows (Morris, 2009; Cieslar et al., 2014). Together with our study, greater arterial AA supply to the mammary gland in NCG-

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Table 3

Blood parameters of lactating dairy cows supplemented without (CON) or with N-carbamoylglutamate (NCG) at 20 g/d.

| Item              | CON       | NCG       | SEM        | P-value |
|-------------------|-----------|-----------|------------|---------|
|                   | T         | W         | T × W      |         |
| AST, U/L          | 69.1      | 63.7      | 1.36       | <0.01   |
| ALT, U/L          | 26.4      | 24.0      | 0.89       | <0.01   |
| NEFA, µmol/L      | 325       | 292       | 30.1       | 0.44    |
| BHB, µmol/L       | 531       | 443       | 38.8       | 0.04    |
| Cholesterol, mmol/L | 3.47   | 3.78      | 0.11       | 0.10    |
| Triglyceride, mmol/L | 0.13   | 0.11      | 0.007      | 0.09    |
| Glucose, mmol/L   | 3.35      | 3.42      | 0.03       | 0.05    |
| Total protein, g/L| 72.0      | 72.3      | 1.02       | 0.84    |
| Creatinine, µmol/L| 89.1      | 85.6      | 2.22       | 0.27    |
| BUN, mmol/L       | 3.76      | 3.37      | 0.13       | 0.01    |
| Albumin (A), g/L  | 30.7      | 30.7      | 0.28       | 0.99    |
| Globulin (G), g/L | 41.3      | 41.7      | 1.05       | 0.72    |
| A-to-G ratio      | 0.77      | 0.75      | 0.02       | 0.67    |
| NO, µmol/L        | 41.5      | 45.8      | 0.88       | <0.01   |
| NO synthase, U/L  | 1,142     | 1,215     | 27.6       | 0.20    |

T = treatment; W = week; T × W = interaction between treatment and week; AST = aspartate aminotransferase; ALT = alanine aminotransferase; NEFA = non-esterified fatty acid; BHB = β-hydroxybutyrate; BUN = blood urea nitrogen; NO = nitric oxide.
Fig. 2. Changes in nitric oxide (NO), NO synthase (NOS; \( P = 0.20; \) T × W interaction, \( P = 0.06 \)), non-esterified fatty acids (NEFA), \( \beta \)-hydroxybutyrate (BHB) and cholesterol in cows supplemented without (CON) or with N-carbamoylglutamate (NCG) at 20 g/d. Bars indicate the SEM. \(* \), \( P < 0.05 \), \( ** \), \( P < 0.10 \).

Table 4
Venous plasma amino acid (AA) concentration, arterial AA supply to mammary gland and mammary plasma flow of AA in dairy cows supplemented without (CON) or with N-carbamoylglutamate (NCG) at 20 g/d.

| Item          | AA concentration\(^1\), \( \mu \)mol/L | SEM | \( P \)-value | Arterial supply\(^2\), \( \times 10^5 \) \( \mu \)mol/d | SEM | \( P \)-value |
|---------------|--------------------------------------|-----|--------------|----------------------------------------|-----|--------------|
| CON          | NCG                                 |     |              |                                        |     |              |
| Arg          | 54.2                                 | 70.4 | 3.18         | \(< 0.01 \)                               | 10.5 | 20.6         | 1.56 | \(< 0.01 \) |
| Thr          | 153                                 | 159  | 6.16         | 0.45                                   | 40.0 | 74.2         | 5.10 | \(< 0.01 \) |
| Val          | 257                                 | 313  | 11.6         | \(< 0.01 \)                             | 38.6 | 87.6         | 7.86 | \(< 0.01 \) |
| Met          | 14.8                                | 19.0 | 1.09         | \(< 0.01 \)                             | 2.9  | 9.3          | 1.18 | 0.01       |
| Ile          | 101                                 | 131  | 7.05         | \(< 0.01 \)                             | 16.7 | 40.6         | 4.00 | \(< 0.01 \) |
| Leu          | 121                                 | 162  | 7.28         | \(< 0.01 \)                             | 22.6 | 51.8         | 4.72 | \(< 0.01 \) |
| Phe          | 50.8                                | 60.9 | 2.13         | \(< 0.01 \)                             | 7.6  | 15.5         | 1.22 | \(< 0.01 \) |
| Lys          | 64.3                                | 79.1 | 3.34         | \(< 0.01 \)                             | 13.2 | 32.4         | 3.50 | \(< 0.01 \) |
| His          | 61.0                                | 64.9 | 2.23         | 0.22                                   | 8.4  | 16.0         | 1.12 | \(< 0.01 \) |
| Essential AA | 877                                 | 1,060 | 34.3        | \(< 0.01 \)                             | 156 | 352          | 27.8 | \(< 0.01 \) |
| Cys          | 29.9                                | 39.0 | 2.29         | \(< 0.01 \)                             | 4.7  | 18.7         | 1.78 | \(< 0.01 \) |
| Glu          | 129                                 | 134  | 6.05         | 0.60                                   | 19.5 | 58.6         | 6.36 | \(< 0.01 \) |
| Tyr          | 60.3                                | 67.3 | 2.48         | 0.05                                   | 9.2  | 17.6         | 1.28 | \(< 0.01 \) |
| Gly          | 418                                 | 397  | 20.2         | 0.46                                   | 57.0 | 101          | 11.4 | 0.06       |
| Ala          | 200                                 | 217  | 7.21         | 0.12                                   | 29.0 | 92.4         | 10.9 | \(< 0.01 \) |
| Ser          | 89.9                                | 97.2 | 4.01         | 0.20                                   | 14.0 | 44.2         | 5.22 | \(< 0.01 \) |
| Pro          | 96.9                                | 122  | 5.57         | \(< 0.01 \)                             | 15.4 | 40.2         | 3.58 | \(< 0.01 \) |
| Asp          | 15.0                                | 15.2 | 0.93         | 0.86                                   | 1.4  | 3.9          | 0.60 | 0.05       |
| Non-essential AA | 1,038                        | 1,088 | 30.5        | 0.23                                   | 150 | 378          | 62.8 | 0.02       |
| Total AA     | 1,911                               | 2,148 | 58.8        | \(< 0.01 \)                             | 256 | 858          | 69.1 | \(< 0.01 \) |
| MPF, L/d     | 11,214                              | 17,383 | 2.556    | 0.04                                   | —   | —            | —   | —          |

MPF = mammary plasma flow.
\(^1\) The concentration of AA in plasma from the coccygeal vein.
\(^2\) The arterial supply of AA to the mammary gland.
cows may be attributed to their higher arterial AA concentrations and MPF. Postpartum dairy cows have increased AA demand, and it is significant to improve the supply of AA by lactogenesis and protein synthesis during the transition period, which is observed in the current study.

On the other hand, nutrient uptake by the mammary gland is a dynamic process with an efficient adaptive mechanism (Wang et al., 2016). When the mammary gland needs more AA, AA clearance rates of the mammary gland increase to support greater milk protein synthesis rates (Rius et al., 2010). Therefore, the higher milk protein yield in the NCG-cows is associated with more proportions of AA cleared in the mammary gland. Additionally, U:O ratio is another important index identifying limiting AA (Lapierre et al., 2012). Both Met and Lys are important limiting AA in dairy cows, thus higher U:O ratios of Met and Lys indicated that more Met and Lys are absorbed and utilized by NCG-cows, leading to a greater lactation performance in these cows. Thus, increased yields of milk and protein in the NCG-cows may be attributed to greater AA supply, MPF, and AA utilization in the mammary gland.

In addition to protein synthesis, Arg, as a precursor for many molecules including proline, creatine and NO is involved in many other physiological functions. Therefore, Arg is considered to be one of the most versatile AA (Wu and Morris, 1998). It is reported that L-Arg reduced ALT and AST, and alleviated the hepatic injury induced by cisplatin in rats (El-Sayed et al., 2019) and by deoxynivalenol in growing pigs (Wu et al., 2013). Therefore, lower plasma activities of ALT and AST in the NCG-cows suggested that these cows were under less hepatic metabolic stress and had better liver function, which contributes to hepatic gluconeogenesis during postpartum milk synthesis (Bell, 1995). In the current study, higher plasma glucose and lower BHB levels with NCG supplementation were consistent with improved gluconeogenesis and liver function. Moreover, the greater glucose of cows supplemented with NCG might have more coenzyme A-SH and Nicotinamide adenine dinucleotide phosphate (NADPH), which are the precursors for fatty acid synthesis in the mammary gland (Zhao, 2014). Palmquist and Conrad (1978) reported that the increased milk fat is accompanied by improved available energy in the body. Liu et al. (2016) found that NCG could alter energy and lipid metabolism, which indicate that NCG is involved in energy and lipid metabolism, and accounted for the increased milk fat yield. Furthermore, previous investigations have reported that improved liver function contributed to the DMI increase (Wu et al., 2009; Li et al., 2016). Therefore, the higher levels of DMI in cows with NCG supplementation may be at least partly due to improved liver function (Zhou et al., 2016a, 2016b). Also, some studies reported that NO increased animal feed intake by inhibiting proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (feed intake-inhibiting neurons) or activating neuropeptide Y (NPY) and agouti-related protein (AgRP) (feed intake-promoting neurons) (Fiakowski and Arzyna, 2010; Wiczer and Thomas, 2010). The greater DMI in cows consuming NCG in our study could be related to the higher levels of NO in plasma. However, no difference in DMI was observed with the addition of NCG in our previous studies (Chacher et al., 2014; Gu et al., 2018). After comprehensively analyzing 35 studies on feeding with the same Met product, Patton (2010) found that the DMI response of cows varied based on the differences in experimental lengths and lactation stages. During the transition period, cows undergo a series of physiological, immunological, and metabolic changes (Zhou et al., 2016b). In the current study conducted in a transition period, the NCG-cows had a greater DMI compared with the CON-cows. This, along with better liver function that was caused by higher Arg and NO concentrations, contributed to improved lactation performance.

## 5. Conclusions

In conclusion, the addition of NCG, initiated from the prepartum stage, is an effective way of improving DMI and milk production during the postpartum period. Moreover, AA supply to the mammary gland, DMI, and production performance of postpartum dairy cows were enhanced as a result of increased plasma Arg and NO and improved liver function.

### Author contributions

Gu Fengfei: methodology, data curation, writing- original draft preparation; Miao Chao: data curation; Jiang Luyi: data curation; Wang Diming: project administration; Liu Hongyun: visualization; Liu jianxin: funding acquisition, supervision, visualization, project administration.

### Conflict of interest

We declare that we have no financial or personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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### Table 5

| Item          | Clearance rate, L/h | SEM  | P-value | U:O ratio | SEM  | P-value |
|---------------|---------------------|------|---------|-----------|------|---------|
| Arg           | 568                 | 508  | 77.6    | 0.60      | 3.16 | 3.02    | 0.26  | 0.80  |
| Thr           | 148                 | 224  | 34.4    | 0.12      | 2.87 | 2.93    | 0.53  | 0.92  |
| Val           | 134                 | 117  | 16.5    | 0.48      | 1.92 | 1.45    | 0.24  | 0.34  |
| Met           | 816                 | 620  | 108     | 0.97      | 1.08 | 1.51    | 0.14  | 0.05  |
| Ile           | 298                 | 404  | 96.4    | 0.44      | 1.86 | 2.31    | 0.29  | 0.45  |
| Leu           | 370                 | 446  | 91.2    | 0.56      | 1.50 | 1.74    | 0.19  | 0.52  |
| Phe           | 216                 | 171  | 27.4    | 0.26      | 0.89 | 1.08    | 0.10  | 0.36  |
| Lys           | 494                 | 572  | 45.2    | 0.24      | 1.32 | 1.87    | 0.15  | <0.01 |
| His           | 155                 | 302  | 79.0    | 0.20      | 1.06 | 1.94    | 0.29  | 0.16  |
| Essential AA  | 442                 | 688  | 164     | 0.30      | 2.67 | 3.07    | 0.31  | 0.53  |
| Cys           | 38.8                | 141  | 22.0    | <0.01     | 1.31 | 4.29    | 0.51  | 0.01  |
| Glu           | 187                 | 226  | 28.4    | 0.34      | 0.47 | 0.61    | 0.06  | 0.29  |
| Tyr           | 170                 | 181  | 14.4    | 0.59      | 1.05 | 0.84    | 0.11  | 0.37  |
| Gly           | 56.4                | 86.8  | 23.4   | 0.36      | 1.72 | 1.77    | 0.19  | 0.90  |
| Ala           | 114                 | 110  | 19.4    | 0.90      | 1.75 | 1.57    | 0.18  | 0.62  |
| Ser           | 202                 | 298  | 57.0    | 0.24      | 0.74 | 1.22    | 0.14  | 0.11  |
| Pro           | 56.8                | 187  | 10.6    | <0.01     | 0.29 | 1.12    | 0.17  | 0.03  |
| Asp           | 128                 | 572  | 12.8    | <0.01     | 0.13 | 0.66    | 0.09  | 0.01  |
| Non-essential AA | 186             | 428  | 47.5    | 0.08      | 1.17 | 2.03    | 0.24  | 0.10  |
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