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Original Research Article

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

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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, whereas plasma glucose ($P = 0.05$) and nitric oxide (NO, $P < 0.01$) concentrations were higher. 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.

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

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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). Chacher et al. (2014) reported that supplementation of NCG could improve nitrogen utilization and tended to increased milk yield in high-yielding dairy cows. Our previous study found that supplemental NCG could improve milk quality but not milk yield in mid-lactation cows (Gu et al., 2018). In both studies, blood concentration of Arg was increased by the NCG supplementation, indicating the effectiveness of NCG in enhancing Arg and milk synthesis in lactating 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 = 0.37), 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 total mixed rations for individual cows. Feeds were offered ad libitum to yield 5% to 10% orts.

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 and orts 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 (milk 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, −1, 0 (the day of calving), 1 (the 7th day 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 −1 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
Corn silage	28.0	19.8
Corn grain, ground	15.4	15.2
Soybean meal	10.3	17.7
Steam-flaked corn		12.7
Sugar beet pulp		6.97
Dicalcium phosphate	0.12	0.51
Sodium bicarbonate	0.50	1.01
Limestone, ground		0.76
Fatty acid calcium		1.50
Mycotoxin binder		0.10
Wheat bran	6.85	
Rice straw	17.9	
Beer grains		1.43
Oat hay	19.9	7.05
Yeast		0.10
Salt	0.17	0.51
Premix[1] (dry period)	0.86	
Premix[2] (lactation period)		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
NE_L[3], MJ/kg DM	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 D_3; ≥380 mg nicotinamide; 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 D_3; 7,000 IU vitamin E; 1,000 mg nicotinamide; 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 reductase method with a NO assay kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China, No. A012-1-2). Total NO synthase was measured with a commercial test kit (Nanjing Jiancheng 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) × 0.965]/Arterial and venous (AV) difference of (Phe + Tyr) (μmol/L)

Arterial free AA supply (μmol/d) = Arterial free AA concentration (μmol/L) × 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) × 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 with repeated measures was used for analysis. Lactation performance and plasma variables were analyzed by considering week, treatment, and interaction 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 PDIFF option of LSMEANS statement. The experimental results were reported as least-squares means. Significance was declared at $P \leq 0.05$, and $0.05 < P \leq 0.10$ 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 × W, $P < 0.01$), and the activity of NO synthase (T × 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
Lactation performance in dairy cows supplemented without (CON) or with N-carbamoylglutamate (NCG) at 20 g/d.

Item	CON	NCG	SEM	*P*-value		
				T	W	T × W
DMI, kg/d	22.3	23.3	0.52	0.06	<0.01	0.72
Yield, kg/d						
Milk	37.7	40.9	0.89	<0.01	<0.01	0.08
ln (ECM)[1]	3.73	3.82	0.02	<0.01	<0.01	0.20
Protein	1.13	1.20	0.02	0.02	<0.01	<0.01
Fat	1.55	1.74	0.04	<0.01	<0.01	0.07
Milk composition, %						
Protein	2.95	2.88	0.05	0.24	<0.01	0.08
Fat	4.04	4.16	0.08	0.27	<0.01	0.06
Lactose	5.02	5.10	0.03	0.06	<0.01	0.05
Total solid	12.4	12.6	0.10	0.10	<0.01	0.34
MUN, mg N/dL	12.9	11.2	0.32	<0.01	<0.01	<0.01
ln (SCC)[1], 10^3/mL	4.20	3.82	0.19	0.12	<0.01	0.22
Feed efficiency[2]	1.88	1.99	0.06	0.13	0.33	0.37

T = treatment; W = week; T × W = interaction between treatment and week; DMI = dry matter intake; ECM = energy-corrected milk yield; MUN = milk urea nitrogen; SCC = somatic cell count.

[1] ECM = 0.3246 × milk yield +13.86 × milk fat yield +7.04 × milk protein yield. ln (ECM) and ln (SCC): the transformed ECM and SCC with natural logarithm, respectively.

[2] Feed efficiency = milk yield (kg/d)/DMI (kg/d).

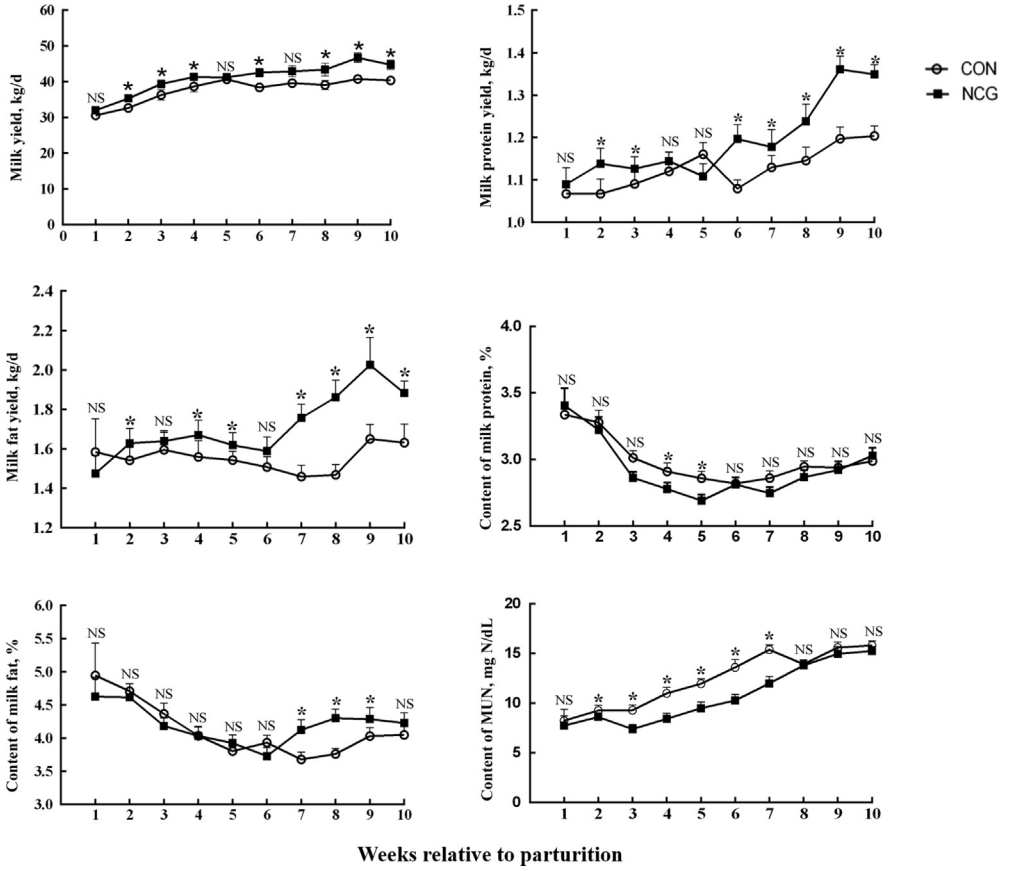

Fig. 1. Changes in yields of milk (A), protein (B), fat (C) and changes in concentrations of milk protein (D), fat (E) and urea nitrogen (MUN, F) in cows supplemented without (CON) or with N-carbamoylglutamate (NCG) at 20 g/d. Error bars indicate the SEM. NS, not significant; *, $P < 0.05$.

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).

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	<0.01	0.51
ALT, U/L	26.4	24.0	0.89	0.03	<0.01	·0.35
NEFA, μmol/L	325	292	30.1	0.44	<0.01	0.94
BHB, μmol/L	531	443	38.8	0.04	<0.01	0.53
Cholesterol, mmol/L	3.47	3.78	0.13	0.10	<0.01	0.01
Triglyceride, mmol/L	0.13	0.11	0.007	0.09	<0.01	0.61
Glucose, mmol/L	3.35	3.42	0.03	0.05	<0.01	0.12
Total protein, g/L	72.0	72.3	1.02	0.84	<0.01	0.90
Creatinine, μmol/L	89.1	85.6	2.22	0.27	<0.01	0.67
BUN, mmol/L	3.76	3.37	0.13	0.01	<0.01	0.47
Albumin (A), g/L	30.7	30.7	0.28	0.99	<0.01	0.58
Globulin (G), g/L	41.3	41.7	1.05	0.72	<0.01	0.81
A-to-G ratio	0.77	0.75	0.02	0.67	<0.01	0.50
NO, μmol/L	41.5	45.8	0.88	<0.01	0.79	0.42
NO synthase, U/L	1,142	1,215	27.6	0.20	0.10	0.06

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.

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 that 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-

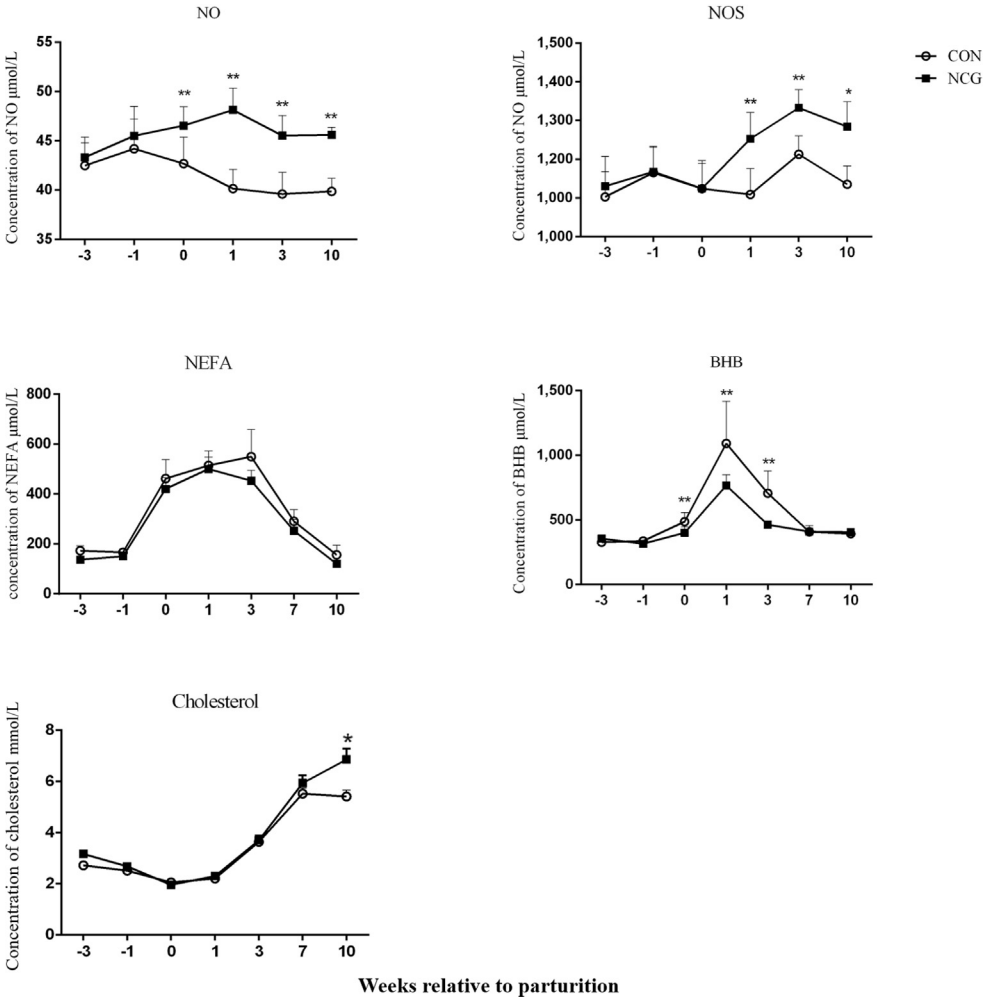

Fig. 2. Changes in nitric oxide (NO), NO synthase (NOS; $P = 0.20$; T × W interaction, $P = 0.06$), non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB) and cholesterol in cows supplemented without (CON) or with N-carbamoylglutamate (NCG) at 20 g/d. Bars indicate the SEM. *, $P < 0.05$, **, $P < 0.10$.

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], μmol/L		SEM	*P*-value	Arterial supply[2], × 10^5 μmol/d		SEM	*P*-value
	CON	NCG			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.

Table 5
The amino acid (AA) clearance rate and uptake-to-output (U:O) ratio of the mammary gland of dairy cows supplemented without (CON) or with N-carbamoylglutamate (NCG) at 20 g/d.

Item	Clearance rate, L/h		SEM	*P*-value	U:O ratio		SEM	*P*-value
	CON	NCG			CON	NCG		
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	820	108	0.97	1.08	1.51	0.14	0.05
Ile	298	404	96.8	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

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) (Fijalkowski 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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References

AOAC. Official methods of analysis. 15th ed. Association of Official Analytical Chemists; 1990.

Batistel F, Arroyo JM, Bellingeri A, Wang L, Saremi B, Parys C, et al. Ethyl-cellulose rumen-protected methionine enhances performance during the periparturient period and early lactation in holstein dairy cows. J Dairy Sci 2017;100:7455–67.
Bell AW. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J Anim Sci 1995;73:2804–19.
Cant JP, DePeters EJ, Baldwin RL. Mammary uptake of energy metabolites in dairy cows fed fat and its relationship to milk protein depression. J Dairy Sci 1993;76:2254–65.
Cao W, Xiao L, Liu G, Fang T, Wu X, Jia G, et al. Dietary arginine and N-carbamoylglutamate supplementation enhances the antioxidant statuses of the liver and plasma against oxidative stress in rats. Food Funct 2016;7:2303–11.
Chacher B, Wang DM, Liu HY, Liu JX. Degradation of L-arginine and N-carbamoylglutamate and their effect on rumen fermentation in vitro. Ital J Anim Sci 2012;11:4693–6.
Chacher B, Zhu W, Ye JA, Wang DM, Liu JX. Effect of dietary N-carbamoylglutamate on milk production and nitrogen utilization in high-yielding dairy cows. J Dairy Sci 2014;97:2338–45.
Cieslar SRL, Madsen TG, Purdie NG, Trout DR, Osborne VR, Cant JP. Mammary blood flow and metabolic activity are linked by a feedback mechanism involving nitric oxide synthesis. J Dairy Sci 2014;97:2090–100.
Ding LY, Chen LM, Wang MZ, Loor JJ, Zhou G, Zhang X, et al. Inhibition of arginase via jugular infusion of Nω-hydroxy-nor-l-arginine inhibits casein synthesis in lactating dairy cows. J Dairy Sci 2018;101:3514–23.
Doepel L, Lapierre H. Deletion of arginine from an abomasal infusion of amino acids does not decrease milk protein yield in Holstein cows. J Dairy Sci 2011;94:864–73.
Edmonson A, Lean I, Weaver L, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. J Dairy Sci 1989;72:68–78.
El-Sayed RM, Ahmed HI, Abd El-Lateef AES, Ali AA. Apoptosis perturbations and expression of regulatory inflammatory factors in cisplatin-depleted rat livers under l-arginine protection. Can J Physiol Pharmacol 2019;97:359–69.
Fijałkowski F, Arzyna R. Role of hypothalamic AMP-activated protein kinase in the control of food intake. Adv Hyg Exp Med 2010;64:231–43 [In Polish with English abstract)].
Gessler P, Buchal P, Schwenk HU, Wermuth B. Favorable long-term outcome after immediate treatment of neonatal hyperammonemia due to N-acetylglutamate synthase deficiency. Eur J Pediatr 2010;169:197–9.
Gu FF, Liang SL, Wei ZH, Wang CP, Liu HY, Liu JX, et al. Short communication: effects of dietary addition of N-carbamylglutamate on milk composition in mid-lactating dairy cows. J Dairy Sci 2018;101:10985–90.
Hanigan MD, France J, Wray-Cahen D, Beever DE, Lobley GE, Reutzel L, et al. Alternative models for analyses of liver and mammarytransorgan metabolite extraction data. Br J Nutr 1998;79:63–78.
Jobgen WS, Fried SK, Fu WJ, Meininger CJ, Wu GY. Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. J Nutr Biochem 2006;17:571–88.
Lapierre H, Lobley GE, Doepel L, Raggio G, Rulquin H, Lemosquet S. Triennial lactation symposium: mammary metabolism of amino acids indairy cows. J Dairy Sci 2012;90:1708–21.
Li C, Batistel F, Osorio JS, Drackley JK, Luchini D, Loor JJ. Peripartal rumen-protected methionine supplementation to higher energy diets elicits positive effects on blood neutrophil gene networks, performance and liver lipid content in dairy cows. J Anim Sci Biotechnol 2016;7:18.
Liu G, Xiao L, Cao W, Fang T, Jia G, Chen X, Zhao H, Wu C, Wang J. Changes in the metabolome of rats after exposure to arginine and N-carbamoylglutamate in combination with diquat, a compound that causes oxidative stress, assessed by HNMR spectroscopy. Food Funct 2016;7:964–74.
Meijer GAL, Meulen JVD, Bakker JGM, Koelen CJVD, Vuuren AMV. Free amino acids in plasma and muscle of high yielding dairy cows in early lactation. J Dairy Sci 1995;78:1131–41.
MOA (Ministry of Agriculture of PR China). Feeding standard of dairy cattle (NY/T 34 - 2004). Beijing, China: China Agricultural Press; 2004.
Morris SM. Recent advance in arginine metabolism: roles and regulation of arginases. Br J Pharmacol 2009;157:922–30.
NRC. Nutrient requirements of dairy cattle. 7 th ed. Washington: Nat. Acad. Press; 2001.
Osorio JS, Ji P, Drackley JK, Luchini D, Loor JJ. Supplemental smartamine m or metasmart during the transition period benefits postpartal cow performance and blood neutrophil function. J Dairy Sci 2013;96:6248–63.
Palmquist DL, Conrad HR. High fat rations for dairy cows. Effects on feed intake, milk and fat production, and plasma metabolites. J Dairy Sci 1978;61:890–901.
Patton RA. Effect of rumen-protected methionine on feedintake, milk production, true milk protein concentration, and truemilk protein yield, and the factors that influence these effects: a meta-analysis. J Dairy Sci 2010;93:2105–18.
Rius AG, Appuhamy JADRN, Cyriac J, Kirovski D, Becvar O, Escobar J, et al. Regulationof protein synthesis in mammary glands of lactating dairy cows by starch and amino acids. J Dairy Sci 2010;93:3114–27.
Sun F, Cao Y, Cai C, Li S, Yu C, Yao Y. Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. PloS One 2016;11:e0160659.
Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97.
Wang B, Sun HZ, Xu NN, Zhu KJ, Liu JX. Amino acid utilization of lactating dairy cows when diets arechanged from an alfalfa-based diet to cereal straw-based diets. Anim Feed Sci Technol 2016;217:56–66.
White HM. The role of TCA cycle anaplerosis in ketosis and fatty liver in periparturient dairy cows. Animals 2015;5:793–802.
Wiczer BM, Thomas G. The role of the mTOR pathway in regulating food intake. Curr Opin Drug Discov Dev 2010;13:604–12.
Wu G, Bazer FW, Davis TA, Kim SW, Li P, Marc Rhoads J, et al. Arginine metabolism and nutrition in growth, health and disease. Amino Acids 2009;37:153–68.
Wu G, Morris SMJ. Arginine metabolism: nitric oxide and beyond. Biochem J 1998;336:1–17.
Wu L, Wang W, Yao K, Zhou T, Yin J, Li T, et al. Effects of dietary arginine and glutamine on alleviating the impairment induced by deoxynivalenol stress and immune relevant cytokines in growing pigs. PloS One 2013;8:e69502.
Yan T, Mayne CS, Patterson DC, Agnew RE. Prediction of body weight and empty body composition using body size measurements in lactating dairy cows. Livest Sci 2009;124:233–41.
Yang SJ, Denbow DM. Interaction of leptin and nitric oxide on food intake in broilers and Leghorns. Physiol Behav 2007;92:651–7.
Zhao FQ. Biology of glucose transport in the mammary gland. J Mammary Gland Biol Neoplasia 2014;19:3–17.
Zhou Z, Vailati-Riboni M, Trevisi E, Drackley JK, Luchini DN, Loor JJ. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. J Dairy Sci 2016a;99:8716–32.
Zhou Z, Bulgari O, Vailati-Riboni M, Trevisi E, Ballou MA, Cardoso FC, et al. Rumen-protected methionine compared with rumen-protected choline improves immunometabolic status in dairy cows during the peripartal period. J Dairy Sci 2016b;99:8956–69.
Zhu J, Zeng XF, Peng Q, Zeng S, Zhao H, Shen H, et al. Maternal N-Carbamylglutamate supplementation during early pregnancy enhances embryonic survival and development through modulation of the endometrial proteome in gilts. J Nutr 2015;145:2212–20.