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## ABSTRACT

The objective of the current study was to investigate the effect of N-carbamoylglutamate (NCG) supplementation on milk production and nitrogen (N) utilization in Chinese Holstein dairy cows. Sixty multiparous cows (78 ± 17.3 d in milk, 635 ± 61.00 kg of body weight, and 41.9 ± 7.9 kg/d milk yield; mean ± SD) were blocked by parity, days in milk, and milk yield and randomly allocated to 1 of 4 groups, each of which was fed a dietary treatment containing 0 (control), 10, 20, or 30 g of NCG/d. Milk yield was recorded weekly. Dry matter intake, milk composition, plasma variables, and urea N contents in plasma, urine, and milk were determined every other week. Blood samples were collected from the coccygeal vein. Rumen microbial protein synthesis was estimated based on the purine derivatives in the urine. Dry matter intake was found to be similar between the treatments. Addition of 20 g of NCG/d tended to increase milk yield (40.2 vs. 38.1 kg/d) and increased the content (2.83 vs. 2.74%) and yield (1.12 vs. 1.02 kg/d) of milk protein compared with the control. The yield and content of milk fat were similar between the treatments, whereas the contents of lactose and total solids increased linearly with an increase in NCG. Dietary supplementation of NCG linearly increased the plasma nitric oxide level and decreased the plasma ammonia N level. Compared with the control, the plasma Arg concentration in cows fed 10, 20, and 30 g of NCG/d was increased by 1.1, 10.4, and 16.0%, respectively. The urea N concentrations in the milk, plasma, and urine decreased with the addition of NCG, although the lowest urea N concentrations were observed with the addition of 20 g of NCG/d. The conversion of dietary crude protein to milk protein exhibited quadratic trends of improvement by NCG supplementation, with a peak at 20 g of NCG/d. The rumen microbial protein synthesis was not altered by NCG supplementation, but the metabolizable protein tended to show a quadratic increase in cows fed 20 g of NCG/d. In conclusion, supplementation of 20 g of NVG/d may alter the plasma metabolites, optimize the AA profile, increase the metabolizable protein utilization, and thereby improve the lactation performance and N utilization of high-yielding dairy cows.
**Key words:** N-carbamoylglutamate, milk production, nitrogen utilization, dairy cow

## INTRODUCTION

Due to economic and environmental considerations, the reduction of N excretion and the improvement of milk production by dairy animals are of interest. High-yielding dairy cows produce a large amount of milk in early to mid lactation and cannot consume sufficient feed to meet the requirements for protein and AA. Supplementation of Lys and Met increased milk yield and N utilization in dairy cows (Wang et al., 2010), whereas the regulatory role of functional AA has not been well known in high-yielding dairy cows. Arginine is one of the functional AA and plays a key role in ureagenesis. As a precursor of polyamines and nitric oxide (NO), Arg can enhance angiogenesis and lactogenesis (Meijer et al., 1985; Morris, 2009).

Supplementation with Arg has been shown to increase milk production in cows (Chew et al., 1984) and sows (Mateo et al., 2008) but results in limited milk protein synthesis in dairy cows (Doepel and Lapierre, 2011). Unprotected Arg is degraded in the rumen and the price of rumen-protected Arg is high; the feeding of rumen-protected Arg appears to be uneconomical (Chacher et al., 2012). Alternatively, N-carbamoylglutamate (**NCG**) is a structural analog of N-acetylglutamate (Gessler et al., 2010), which is a cofactor of carbamoyl phosphate synthetase-1, a rate-liming enzyme responsible for both the urea cycle and the Arg synthetic pathway (Wu et al., 2004). Therefore, NCG is considered an Arg enhancer. In previous studaa NCG 28(udanta

**Table 1.** Ingredients and chemical composition of the experimental diet

| Composition | Amount |
|---|---|
| Ingredient composition, % of DM | |
| Corn silage | 24.6 |
| Alfalfa hay | 14.0 |
| Chinese wild ryegrass | 5.70 |
| Cottonseed meal | 3.00 |
| Sugar beet pulp | 3.80 |
| Corn grain | 15.10 |
| Wheat bran | 2.20 |
| Barley | 5.27 |
| Extruded soybeans | 2.30 |
| Soybean meal | 7.7 |
| Whole cottonseeds | 5.30 |
| Dried distillers grains with solubles | 2.00 |
| Soybean curb residue | 0.81 |
| Rapeseed meal | 3.5 |
| Premix[1] | 0.51 |
| Salt | 0.51 |
| Dicalcium phosphate | 0.60 |
| $CaCO_3$ | 0.50 |
| Sodium bicarbonate | 0.70 |
| Ca salts of long-chain FA | 1.80 |
| Coated urea | 0.11 |
| Methionine | 0.04 |
| Total | 100.0 |
| Chemical composition | |
| DM, % | 53.5 |
| CP, % of DM | 17.6 |
| RDP, % of CP | 62.5 |
| ADF, % of DM | 24.8 |
| NDF, % of DM | 33.3 |
| NFC,[2] % of DM | 37.1 |
| Ca,[3] % | 1.10 |
| P,[3] % | 0.50 |
| $NE_L$,[3] Mcal/kg of DM | 1.65 |
| Arg, % | 0.73 |
| Lys, % | 0.80 |
| Met, % | 0.27 |
| Lys:Met | 2.96:1 |

[1]Formulated to provide (per kilogram of premix) 1,000,000 IU of vitamin A, 200,000 IU of vitamin D, 1,250 IU of vitamin E, 14,000 mg of Zn, 100 mg of SE, 180 mg of I, 3,000 mg of Fe, 40 mg of Co, 3,000 mg of Mn, and 3,000 mg of Cu.

[2]Calculated as 100 − (% NDF + % CP + % ether extract + % ash).

[3]Calculated based on data from the Ministry of Agriculture of P. R. China (MOA, 2004).

Dietary NCG supplementation has been observed to increase the endogenous synthesis of Arg and the muscle protein synthesis in piglets (Wu et al., 2004; Frank et al., 2007). Ammonia N in rumen epithelial and duodenum mucosal cells can be utilized through the ornithine-urea cycle through supplemented NCG if carbamoyl phosphate synthetase-1 is activated (Oba et al., 2005). Milk protein synthesis has been found to be highly correlated with the urea cycle and dietary AA composition (Lobley et al., 1995). Therefore, the N utilization and lactation performance of high-yielding cows can be improved by efficiently enhancing urea synthesis in the gut and balancing the available AA profile to closely match the AA composition required for milk synthesis.

However, limited information is available on the efficacy of NCG in dairy cows. The purpose of this study was to investigate the effect of NCG supplementation on the DMI, milk production, and N utilization efficiency in high-yielding Chinese Holstein dairy cows fed an isonitrogenous and isocaloric basal diet.

## MATERIALS AND METHODS

### *Animals, Diet, and Experimental Design*

The use of the animals was approved by the Animal Care Committee of Zhejiang University and Xinghuo Dairy Farm, which is affiliated with Shanghai Bright Holstein Co. Ltd. (Qiqihar, China). Sixty high-yielding Chinese Holstein cows (parity = 3.35 ± 1.2; DIM = 78 ± 17.3; milk yield = 41.9 ± 7.9 kg/d; mean ± SD) were selected for the feeding trail. The animals were divided into 15 blocks based on parity, milk yield, and DIM and randomly allocated into 4 groups. The cows were fed 0 (control), 20, 40, or 60 g/d of a treatment mix, which was a 50:50 mix of sodium glutamate and corn starch prepared by Hangzhou King Techina Technology Co. Ltd. (Hangzhou, China). Thus, the actual additional amounts of NCG were 0, 10, 20, or 30 g/d, respectively.

The cows were housed in a tie-stall barn and fed isonitrogenous and isocaloric basal diets throughout the experimental period. The ingredients and nutrient composition of the experimental diet are presented in Table 1. The supplemental NCG was added at an equal amount twice per day at 0600 and 1800 h by top-dress feeding onto the TMR for individual cows. The cows had free access to drinking water, were milked 3 times per day, and were fed at 0600, 1400, and 2000 h daily. The feeds were offered ad libitum to yield 5% orts. The feeding trial was composed of a 10-d adaptation period and 7-wk experimental periods.

### *Sampling, Measurement, and Analyses*

The feeds that were offered and refused were recorded for 2 consecutive days, and representative samples of the TMR and orts were collected on the same day and the same time every other week throughout the experimental period to calculate the DMI (Wang et al., 2010). The DMI determination started from the first week of the study. The sample preparation and analysis were performed using previously described methods (Zhu et al., 2013). All of the samples were analyzed for NDF and ADF (Van Soest et al., 1991), DM and total N (method 988.05; AOAC, 1990).

The milk yield was recorded (Waikato Milking Systems NZ Ltd., Waikato, Hamilton, New Zealand) 3 times per day on the fourth day of each week. Milk samples were collected on the fourth day of wk 1, 3, 5, and 7 through milking at 0600, 1400, and 2000 h; these samples were collected in two 50-mL aliquots at a proportion of 4:3:3, respectively. One aliquot was mixed with bronopol (milk preservative; D & F Control Systems Inc., San Ramon, CA) and used to determine the milk fat, protein, and lactose contents through infrared analysis (Laporte and Paquin, 1999) using a 4-channel spectrophotometer (MilkoScan; Foss Electric A/S, Hillerød, Denmark) and SCC using a cell counter (Fossomatic 400; Foss Electric A/S). The other aliquot was stored at −20°C for analysis using the MUN assay (Rahmatullah and Boyde, 1980).

Blood samples (8 mL) were collected from the coccygeal vein of each cow into a 10-mL tube containing anticoagulant (heparin) approximately 3 h after feeding on the fifth day of wk 1, 3, 5, and 7; and then centrifuged at 3,000 × *g* for 15 min at 4°C to collect the plasma. The plasma was frozen at −20°C for subsequent analysis of BUN (Wang et al., 2007), total protein (Thomas, 1998), albumin (Johnson et al., 1999), NEFA (McCutcheon and Bauman, 1986), triglyceride (Cole et al., 1997), glucose (Barham and Trinder, 1972), and BHBA (Moss and Henderson, 1999). The plasma concentration of NO was measured calorimetrically (Green et al., 1982) using the Griess reaction with a commercial NO test kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China). The concentrations of total AA in the plasma were analyzed according to the procedure described by Calder et al. (1999) using kits (DiaSys Diagnostic Systems; Shanghai Co. Ltd., Shanghai, China). The plasma ammonia N concentration was determined by the method developed by Seligson and Hirahara (1957).

At the beginning and end of the experiment, all the cows were weighed before the morning feeding to calculate the BW change. The scaled score (1, 2, 3, 4, and 5) was used to determine the BCS according to a standard procedure (Edmonson et al., 1989).

### Microbial Protein and MP

The MP supply was calculated as the sum of the intestinally absorbable dietary protein (**IADP**) and the intestinally absorbable microbial CP (**IAMCP**). The IADP was estimated by using the following equation: IADP = RUP × CP intake × the intestinal digestibility of RUP (**IDP**). The methods used for the determination of IDP, RUP, and microbial CP (**MCP**) was described previously (Zhu et al., 2013). Briefly, the IDP was determined according to the modified 3-step procedure developed by Gargallo et al. (2006). To determine the RUP, the nylon bag technique was used, and a passage rate of 5% was assumed to calculate the effective rumen degradability (Krizsan et al., 2010).

The MCP synthesis in the rumen was estimated by the urinary purine derivative value, as reported by Chen and Gomes (1992). The collection and pretreatment of the urine samples were performed using the method described by Zhu et al. (2013). Briefly, spot urine samples (about 300 mL per sampling) were collected for 2 consecutive days every other week. Aliquots of urine samples were immediately acidified with 2*M* $H_2SO_4$ to a pH <3.0, diluted 1:10 with distilled water, and stored frozen at −20°C. The IAMCP value was estimated as the MCP yield × 0.64 (NRC, 2001).

### Statistical Analysis

All the data were analyzed through the mixed-model procedure of SAS (SAS Institute, 2000) using the covariance type AR(1) for repeated measures. A randomized block design with repeated measurements was used, with week, treatment, the interaction treatment × week, and block as the main effects, and cow within diet as a random effect. The linear and quadratic effects of the treatment on all of the variables were tested through orthogonal polynomial contrast. The effect of the week was included as a repeated measure. The means were separated using the PDIFF option in the LSMEANS treatment. The results are reported as least squares means. Significance was declared at $P \leq 0.05$, and tendency was declared at $0.05 < P \leq 0.10$.

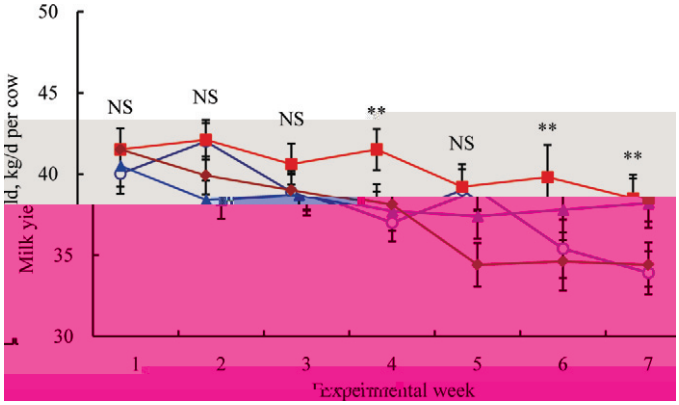


**Figure 1.** Changes in the milk yield in the control cows (○) and in the cows supplemented with 10 (▲), 20 (■), or 30 g/d (◆) of N-carbamoylglutamate (NCG). Error bars indicate the SE. ** = Significant difference between control and 20 g/d NCG supplementation. Color version available in the online PDF.

**Table 2.** Effect of dietary N-carbamoylglutamate (NCG) on the milk yield and milk composition of Chinese Holstein dairy cows

| | NCG, g/d per cow | | | | *P*-value | |
|---|---|---|---|---|---|---|
| Item | 0 | 10 | 20 | 30 | Linear | Quadratic |
| DMI, kg/d | 22.4 | 22.8 | 22.9 | 22.6 | 0.55 | 0.15 |
| Yield, kg/d | | | | | | |
| Milk | 38.1 | 38.1 | 40.2 | 37.3 | 0.98 | 0.07 |
| 4% FCM | 34.3 | 34.7 | 36.5 | 34.2 | 0.70 | 0.15 |
| Milk composition, % | | | | | | |
| Protein | 2.74 | 2.75 | 2.83 | 2.74 | 0.45 | 0.03 |
| Fat | 3.37 | 3.36 | 3.44 | 3.46 | 0.32 | 0.84 |
| Lactose | 4.95 | 4.92 | 4.98 | 5.00 | 0.05 | 0.22 |
| TS | 12.1 | 12.0 | 12.4 | 12.2 | 0.08 | 0.74 |
| SCC, $\times 10^3$/mL | 93.9 | 52.8 | 86.4 | 81.0 | 0.93 | 0.24 |
| Feed efficiency[1] | 1.53 | 1.47 | 1.59 | 1.53 | 0.54 | 0.97 |

[1]Feed efficiency = milk yield/DMI.

## RESULTS

### DMI and Milk Performance

The results of the DMI and milk performance measurements are shown in Table 2. The DMI was not significantly altered by NCG supplementation. Supplementation of NCG tended to result in a greater milk yield ($P = 0.07$, quadratic), which peaked at 20 g of NCG/d (Figure 1), and TS content ($P = 0.08$, linear). The cows administered 20 g/d NCG supplementation exhibited an increased milk protein yield ($P < 0.01$) and an increased protein content ($P = 0.03$). The addition of 20 and 30 g of NCG/d increased ($P < 0.05$, linear) the milk lactose content but did not alter the yield of lactose throughout the treatments. The yield and content of milk fat and the SCC were not different between any of the treatments. The 4% FCM and feed efficiency were not affected by dietary NCG supplementation, although a numerically greater FCM yield was observed in cows fed 20 g of NCG/d compared with the control (36.5 vs. 34.4 kg/d).

### Plasma Metabolite and AA Profiles

The plasma concentrations of total protein, albumin, triglyceride, BHBA, glucose, and NEFA were similar between the different treatments (Table 3). The dietary NCG increased ($P < 0.01$, linear) the plasma NO concentration and decreased ($P < 0.01$, linear) the plasma ammonia N concentration. No significant effects of NCG supplementation were found on other plasma parameters.

The effects of NCG supplementation on the plasma AA are presented in Table 4. All of the essential (**EAA**) and nonessential AA (**NEAA**) except Cys were significantly altered by NCG supplementation. The percentage increases in the plasma Arg concentration in cows fed 10, 20, and 30 g of NCG/d were 1.13, 10.4, and 16.0, respectively. Supplementation of NCG increased ($P < 0.01$, linear and quadratic) the plasma concentration of Met, with the peak at 20 g of NCG/d, whereas the concentration of Lys was decreased ($P < 0.01$, linear and quadratic), with the nadir at 30 g of NCG/d. The resulting Lys:Met ratios in the plasma of

**Table 3.** Effects of dietary N-carbamoylglutamate (NCG) on the blood parameters of Chinese Holstein dairy cows

| | NCG, g/d per cow | | | | *P*-value | |
|---|---|---|---|---|---|---|
| Item | 0 | 10 | 20 | 30 | Linear | Quadratic |
| Total protein, g/L | 84.3 | 86.6 | 87.5 | 84.8 | 0.83 | 0.38 |
| Albumin, g/L | 42.7 | 42.6 | 41.19 | 43.1 | 0.96 | 0.37 |
| Glucose, mmol/L | 3.40 | 3.39 | 3.43 | 3.52 | 0.18 | 0.47 |
| Triglyceride, mmol/L | 0.12 | 0.12 | 0.11 | 0.12 | 0.38 | 0.34 |
| Globulin, g/L | 41.7 | 43.7 | 47.5 | 44.2 | 0.55 | 0.53 |
| NEFA, μmol/L | 120 | 138 | 131 | 126 | 0.66 | 0.16 |
| BHBA, μmol/L | 615 | 651 | 610 | 537 | 0.21 | 0.26 |
| Nitric oxide, μmol/L | 25.1 | 29.1 | 44.3 | 41.0 | <0.01 | 0.45 |
| Ammonia N, μmol/L | 1,288 | 942.2 | 599.8 | 595.1 | <0.01 | 0.05 |

cows fed 0, 10, 20, and 30 g of NCG/d were 3.82, 3.88, 3.18, and 3.30, respectively. The total EAA decreased ($P < 0.01$, linear and quadratic), with the nadir at 30 g of NCG/d, and the total NEAA increased ($P < 0.01$, linear and quadratic), with the peak at 20 g of NCG/d.

### MP Supply and N Utilization Efficiency

Supplementation of NCG did not alter MCP synthesis estimated from purine derivatives. The IAMCP and IADP did not differ between the different treatments, but the MP tended to increase ($P = 0.06$) in cows fed 20 g of NCG/d compared with the control.

The concentrations of BUN and MUN were decreased ($P = 0.03$, linear) in cows that received NCG supplementation, whereas the urea N concentration in the urine was decreased ($P < 0.03$, quadratic), with the nadir in cows fed 20 g of NCG/d. The conversion of dietary CP to milk protein exhibited quadratic trends ($P = 0.07$) of improvement by NCG supplementation, with a peak at 20 g of NCG/d.

No statistical significance was observed in BW change among different treatments, but the change in the BCS ($P = 0.05$, linear) was greater for cows supplemented with 20 g of NCG/d.

## DISCUSSION

### Lactation Performance

The lactation performance of dairy cows was improved quadratically with an increasing level of NCG supplementation and peaked at 20 g of NCG/d (Table 2). The increases in the milk yield and milk protein yield may be associated with many factors, including a balanced plasma AA profile and an alteration in the plasma ammonia N and NO through efficient ureagenesis. Balancing the EAA profile of dairy cows through diets can improve the efficiency of the conversion of MP

**Table 5.** Effects of dietary N-carbamoylglutamate (NCG) on microbial CP (MCP), N utilization efficiency, and urea N concentration in blood plasma, urine, and milk

| | NCG, g/d per cow | | | | *P*-value | |
|---|---|---|---|---|---|---|
| Item | 0 | 10 | 20 | 30 | Linear | Quadratic |
| MCP,[1] g/d | 1,316 | 1,327 | 1,394 | 1,307 | 0.79 | 0.16 |
| MP supply, g/d | | | | | | |
| IAMCP[2] | 842 | 849 | 892 | 836 | 0.79 | 0.16 |
| IADP[3] | 1,776 | 1,793 | 1,802 | 1,788 | 0.64 | 0.51 |
| MP[4] | 2,623 | 2,676 | 2,707 | 2,629 | 0.74 | 0.06 |
| N conversion[5] | 0.261 | 0.264 | 0.289 | 0.262 | 0.40 | 0.07 |
| Urea N, mg/dL | | | | | | |
| Milk | 12.4 | 11.8 | 11.5 | 11.7 | 0.03 | 0.13 |
| Plasma | 7.06 | 7.10 | 6.24 | 6.63 | 0.03 | 0.50 |
| Urine | 1,071 | 921.6 | 794.0 | 950.7 | 0.12 | 0.03 |
| BW change, g/d | 54.4 | 43.9 | 117.0 | 77.7 | 0.11 | 0.47 |
| BCS change | 0.10 | 0.09 | 0.25 | 0.14 | 0.05 | 0.16 |

[1]MCP = microbial CP calculated based on purine derivatives (PD).

[2]IAMCP = intestinally absorbable MCP = MCP × 0.64 (NRC, 2001).

[3]IADP = intestinally absorbable dietary protein = RUP × CP intake × IDP, where IDP is the measured intestinal digestibility of RUP. The feedstuff incubated in the rumen for 16 h was used to determine the IDP according to a modified 3-step procedure (Gargallo et al., 2006).

[4]MP = IAMCP + IADP.

[5]Milk protein yield/CP intake.

Arg has been found to increase milk production in cows (Chew et al., 1984) and sows (Mateo et al., 2008). In our study, the addition of 10, 20, and 30 g of NCG/d to cows increased their plasma Arg concentration by 1.13, 10.4, and 16.0%, respectively, compared with the control (Table 3). This finding suggests that 10 g/d NCG supplementation may not be sufficient to stimulate the endogenous synthesis of Arg. Interestingly, the plasma Arg concentration was greater in the cows fed 30 g of NCG/d, but the milk production was greater in the cows fed 20 g of NCG/d. This discrepancy may be attributed to the more optimal MP and MCP obtained in the cows fed 20 g of NCG/d. Additionally, NCG supplementation increased the NO concentration in the plasma, which might enhance the mammary blood flow and the nutrient availability for milk synthesis, as was reported by Renaudeau et al. (2002).

These results suggest that dietary supplementation of 20 g of NCG/d is potentially an alternative to Arg supplementation for the improvement of milk protein synthesis because it efficiently alters the Arg family of AA and balances the limited AA profile in the plasma, which might improve the lactation performance of high-yielding dairy cows.

### *N Utilization, MCP Synthesis, and MP Utilization*

High dietary CP levels are positively associated with increased ammonia concentrations and have been shown to decrease the efficiency of N utilization for milk production (Hristov et al., 2004). In dairy cows, the urea N in blood, urine, and milk is mainly derived from excessive absorbed ammonia and the deamination of AA or MP (Wang et al., 2008). The addition of 20 g of NCG/d decreased ($P = 0.03$) the urea N concentrations in plasma, milk, and urine compared with that of the control, resulting in a greater ($P < 0.07$) N conversion efficiency, as calculated by the ratio of the milk protein yield to the CP intake (Table 5). Davenport et al. (1990) observed improved N retention and lower plasma ammonia N concentrations in growing heifers that were abomasally administered Arg. Arginine is an important AA related to the transportation, storage, and excretion of N and the disposition of ammonia via the urea cycle (Barbul, 1986). In our study, the addition of NCG enhanced the Arg content (Table 3), reduced the disposal of N in the urine (Table 5), and resulted in the capture of more N as MCP and milk protein. This result indicates that NCG might improve urea synthesis and consequently contributes to improved MP utilization. The results from our study are consistent with the reports from Oba et al. (2005), who found that rumen epithelial cells and duodenal cells have the capacity to utilize NCG for urea N recycling. Thus, urea synthesis through NCG in ruminant gut tissues might reduce ammonia absorption and improve N utilization. The efficient conversion of N by the addition of NCG to dairy cow ratios containing high CP (17.6%) may also reduce the environmental impact of livestock production.

## CONCLUSIONS

Supplementation of NCG to dairy cows through diet may alter the plasma metabolite profiles and optimize

the AA profile through efficient urea cycle regulation. In the present study, the optimal supplementation level of NCG was determined to be 20 g/d, based on the improved milk performance and N utilization efficiency obtained in high-yielding Chinese Holstein dairy cows that were fed a protein-rich diet during early to mid lactation. Additional studies may be warranted to evaluate the effect of NCG on the growth and reproduction performance of dairy cows.

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