Effects of Oral Administration of N-Carbamylglutamate on Growth Performance, Blood Parameters, and Organ Weights in Suckling Goat Kids (Postprint)

Authors: Wang Ruocheng, Ji Yu, Sun Lingwei, Mao Dagan, Wang Jingang, Ma Tie-Wei, Wang Feng, Wang Ziyu

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Abstract

This experiment aimed to investigate the effects of oral administration of N-carbamylglutamate (NCG) on growth performance, blood parameters, and organ weights in 1- to 41-day-old suckling goat kids. Thirty-two suckling goat kids with similar body weight [(3.1±0.3) kg] at 1 day of age were selected and randomly divided into 2 groups (16 kids per group), receiving daily oral administration of 0 (control) or 100 mg/kg BW of NCG. The kids were slaughtered at 41 days of age. The results showed that, compared with the control group: 1) NCG administration significantly increased the average daily gain from 1 to 41 days of age and significantly reduced the diarrhea rate ($P<0.05$); 2) NCG administration significantly increased plasma total protein and albumin contents in 41-day-old kids ($P<0.05$), while significantly decreasing plasma ammonia and urea nitrogen contents in 21- and 41-day-old kids ($P<0.05$); 3) NCG administration significantly increased plasma insulin, growth hormone, and nitric oxide contents in 21- and 41-day-old kids ($P<0.05$); 4) NCG administration significantly increased plasma arginine, ornithine, and citrulline contents ($P<0.05$); 5) NCG administration significantly increased the relative weights of the spleen, small intestine, and large intestine in kids ($P<0.05$). These results suggest that oral administration of NCG promotes growth and development and endogenous arginine synthesis in suckling goat kids.

Full Text

Effects of Oral N-Carbamylglutamate Supplementation on Growth Performance, Blood Parameters and Organ Weight of Suckling Kidlets

WANG Ruocheng[1], JI Yu[1], SUN Lingwei[1], MAO Dagan[1], WANG Jingang[2], MA Tiewei[1], WANG Feng[1,2], WANG Ziyu[1,2*]

[1]College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China
[2]Research Centre of Haimen Goats, Nanjing Agricultural University, Haimen 216121, China

Abstract: This study investigated the effects of oral N-carbamylglutamate (NCG) supplementation on growth performance, blood parameters and organ weight in suckling kidlets from 1 to 41 days of age. Thirty-two suckling kidlets with similar birth weight [(3.1±0.3) kg] were randomly assigned to two groups (16 kidlets per group) at 1 day of age. The kidlets were orally administered NCG at daily doses of 0 (control) or 100 mg/kg BW. All kidlets were slaughtered at 41 days of age. The results showed that compared with the control group: 1) NCG supplementation significantly increased average daily gain and reduced diarrhea rate during days 1–41 ($P<0.05$); 2) NCG supplementation significantly increased plasma total protein and albumin contents at 41 days of age ($P<0.05$), while significantly decreasing plasma ammonia and urea nitrogen contents at 21 and 41 days of age ($P<0.05$); 3) NCG supplementation significantly increased plasma insulin, growth hormone and nitric oxide contents at 21 and 41 days of age ($P<0.05$); 4) NCG supplementation significantly increased plasma arginine, ornithine and citrulline contents ($P<0.05$); and 5) NCG supplementation significantly increased the relative weights of spleen, small intestine and large intestine ($P<0.05$). These results indicate that oral NCG supplementation promotes growth performance and endogenous arginine synthesis in suckling kidlets.

Keywords: N-carbamylglutamate; suckling kidlet; growth performance; blood parameters; organ weight

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Arginine is an essential amino acid for maintaining optimal growth and nitrogen balance in young livestock, playing crucial roles in promoting intestinal protein synthesis, enhancing intestinal immunity and antioxidant capacity, and maintaining intestinal health. However, the arginine content in maternal milk combined with endogenous synthesis often fails to meet the nutritional requirements of young animals, potentially limiting their growth performance, thus necessitating additional supplementation. Direct arginine supplementation is limited by its high degradation rate in the rumen and the high cost of rumen-protected arginine, restricting its application in the feed industry.

N-carbamylglutamate (NCG), a structural analog of N-acetylglutamate (NAG)

which activates arginine synthesis, promotes intestinal arginine synthesis by enhancing the activity of carbamoyl phosphate synthetase-I (CPS-I), the rate-limiting enzyme in arginine synthesis. Consequently, NCG is considered an arginine enhancer. Unlike arginine, NCG has an extremely low degradation rate in the rumen. Furthermore, unlike NAG, NCG is not degraded by highly active deacylases in mammalian cytoplasm, a characteristic that facilitates its entry into mitochondrial to exert its effects. Oral NCG administration in suckling piglets has been shown to significantly increase plasma arginine and growth hormone (GH) contents, promoting piglet growth and skeletal muscle protein synthesis. NCG administration also promotes growth performance and endogenous arginine synthesis in 7-day-old suckling piglets. However, few studies have reported the effects of NCG on growth performance in suckling goat kidlets. This experiment aimed to investigate the effects of oral NCG supplementation on growth performance, blood parameters and organ weight in suckling kidlets, providing a theoretical basis for NCG application in suckling kidlets.

Materials and Methods

Experimental Animals and Design

Thirty-two healthy, 1-day-old crossbred male kidlets (Boer × Haimen) with similar birth weight were selected as experimental animals. To exclude potential effects of ewe milk yield and number of suckling kidlets on growth performance, each ewe nursed two male kidlets, with the two kidlets from each ewe assigned to different groups (16 kidlets per group). From 1 to 41 days of age, kidlets were orally administered NCG at doses of 0 (control) or 100 mg/kg BW daily for 40 days. NCG was administered twice daily (morning and evening) by mixing with warm water and slowly injecting into the kidlets' mouths using a 5 mL syringe; the control group received an equal volume of warm water. NCG was purchased from Asia-Pacific Xingmu Technology Co., Ltd. (production license number: Yusi Tian (2014) T05005) with a purity of 97%.

Feeding Management and Sample Collection

The experiment was conducted at the Research Centre of Haimen Goats, Nanjing Agricultural University. The diet of lactating ewes was formulated according to NRC (2007) recommendations for lactating sheep. The composition and nutrient levels of the basal diet are shown in . During the experimental period, lactating ewes were individually housed with ad libitum access to water and feed. Kidlets suckled freely. Deworming and vaccination were performed according to farm management practices. At 1, 21 and 41 days of age, five kidlets were randomly selected from each group and 5 mL of blood was collected from the jugular vein 2 hours after morning NCG administration into sterile heparinized tubes. Plasma was immediately separated by centrifugation at 3,000 r/min for 8 min and stored at -20°C.

Growth Performance

Kidlets in each group were weighed once at 1, 21 and 41 days of age after a 12-hour fasting period. To ensure fasting status, kidlets were separated from their dams immediately after blood collection on weighing days and weighed after 12 hours. Average daily gain was calculated using the following formula:

Average daily gain (kg/d) = (final weight - initial weight) / number of days.

Diarrhea Rate Determination

The number of diarrheic kidlets was recorded daily. Diarrhea was defined as soft or watery feces with fecal contamination of the tail region. Diarrhea rate was calculated using the following formula:

Diarrhea rate (%) = 100 × number of diarrheic episodes / (total number of kidlets × experimental days).

Plasma Biochemical Indices Determination

Plasma samples collected at 1, 21 and 41 days of age were thawed at 4°C and analyzed using a Vital Scientific clinical chemistry analyzer to determine glucose (GLU), total protein (TP), albumin (ALB), urea nitrogen (UN), ammonia, triglyceride (TG) and total cholesterol (TC) contents.

Plasma Hormone Content Determination

Plasma samples collected at 41 days of age were thawed at 4°C. Plasma GH, insulin (Ins) and nitric oxide (NO) contents were determined using ELISA kits (purchased from Shanghai Kaimaishu Biotechnology Co., Ltd.).

Plasma Amino Acid Content Determination

Plasma samples collected at 41 days of age were thawed at 4°C. For analysis, 0.3 mL of plasma was diluted 1:3 with 10% sulfosalicylic acid, mixed thoroughly, and centrifuged at 12,000 r/min for 30 min at room temperature. The supernatant was collected and amino acid contents were determined using a Hitachi L-8800 automatic amino acid analyzer.

Statistical Analysis

Experimental data were analyzed using SPSS 19.0 software. Differences between groups were tested using t-tests, and percentage data were analyzed using ^{2} tests. Data (except percentages) are expressed as mean ± standard deviation, with $P<0.05$ considered statistically significant. A few kidlets died or were culled during the experiment, resulting in 13 and 14 kidlets in the control and NCG groups, respectively, for statistical analysis.

Results

Effects of Oral NCG Supplementation on Growth Performance and Diarrhea Rate in Suckling Kidlets

As shown in , compared with the control group, NCG supplementation increased 41-day body weight by 0.89 kg ($P<0.05$) and significantly improved average daily gain during days 21–41 and days 1–41 ($P<0.05$). However, NCG had no significant effect on 21-day body weight or average daily gain during days 1–21 ($P>0.05$). The diarrhea rate was significantly lower in the NCG group compared with the control group ($P<0.05$).

Effects of Oral NCG Supplementation on Plasma Biochemical Indices in Suckling Kidlets

As shown in , plasma biochemical indices at 1 day of age did not differ significantly between groups ($P>0.05$). The NCG group had significantly higher plasma TP and ALB contents at 41 days of age compared with the control group ($P<0.05$). Conversely, plasma ammonia and UN contents at 21 and 41 days of age were significantly lower in the NCG group ($P<0.05$).

Effects of Oral NCG Supplementation on Plasma Hormones and NO Content in Suckling Kidlets

As shown in , plasma hormone indices at 1 day of age did not differ significantly between groups ($P>0.05$). Compared with the control group, plasma GH, Ins and NO contents at 21 and 41 days of age were significantly higher in the NCG group ($P<0.05$).

Effects of Oral NCG Supplementation on Plasma Amino Acid Content in Suckling Kidlets

As shown in , plasma arginine, citrulline and ornithine contents were significantly higher in the NCG group compared with the control group ($P<0.05$).

Effects of Oral NCG Supplementation on Organ Relative Weight in Suckling Kidlets

As shown in , NCG supplementation increased the relative weight of all organs, with the relative weights of spleen, small intestine and large intestine being significantly higher in the NCG group compared with the control group ($P<0.05$).

Discussion

Multiple studies have demonstrated that arginine is an essential amino acid for young livestock, likely because the arginine provided by maternal milk and synthesized endogenously cannot meet nutritional requirements for maximal growth. While direct arginine supplementation can increase plasma arginine

content, it may cause absorption disorders of other amino acids (tryptophan, lysine and histidine) and excessive NO production, which can damage tissues. Regulating endogenous arginine content to improve growth performance can avoid these negative effects. NAG is a necessary cofactor for endogenous arginine synthesis, and NCG, as a structural analog of NAG, promotes arginine synthesis in various mammals including rodents, pigs, cattle and sheep.

Previous studies on NCG effects in young livestock have focused primarily on piglets. For example, oral NCG administration promotes growth performance and endogenous arginine synthesis in suckling piglets, while dietary supplementation with 0.08% NCG significantly improves growth performance, promotes intestinal development, alleviates weaning stress and reduces diarrhea rate in weaned piglets. However, few studies have examined NCG effects in newborn ruminants. The present study demonstrated that 40 days of NCG supplementation significantly increased average daily gain and reduced diarrhea rate in suckling kidlets, possibly by promoting endogenous arginine synthesis and subsequently enhancing skeletal muscle protein synthesis and intestinal development. These findings are consistent with reports that NCG supplementation increases average daily gain and skeletal muscle protein synthesis in suckling piglets, and that dietary arginine supplementation promotes intestinal development and reduces diarrhea rate in piglets.

Blood ammonia is a toxic metabolite that animals convert to urea through the urea cycle, with CPS-I being the key enzyme in this conversion process. NAG is an allosteric activator of CPS-I, and as its analog, NCG can promote the conversion of excess ammonia to carbamoyl phosphate and subsequently to urea. In this study, NCG supplementation significantly reduced plasma ammonia and UN contents at 21 and 41 days of age while increasing plasma TP content, indicating that NCG activated CPS-I to convert excess ammonia into urea while promoting protein synthesis and nitrogen retention.

Albumin is an important indicator of stress, typically decreasing under stress conditions. The significantly higher plasma ALB level at 41 days of age in NCG-supplemented kidlets suggests that NCG improved their stress resistance. Arginine is known to promote the release of endogenous insulin and GH, and this study found that NCG supplementation significantly increased plasma GH and Ins levels at 21 and 41 days of age, likely due to increased plasma arginine levels. Arginine is also a precursor and regulator of NO synthesis. NCG supplementation significantly increased plasma NO levels, but these remained within the normal physiological range, indicating that NCG does not cause excessive NO production. The moderate increase in NO can regulate immunity, promote angiogenesis and reduce gastrointestinal mucosal damage, thereby promoting kidlet growth.

One week after birth, the arginine provided by sow milk and synthesized endogenously cannot meet the requirements for maximal growth in piglets. While high-dose arginine or citrulline supplementation can increase plasma arginine content, it may reduce plasma contents of other essential amino acids (lysine

and tryptophan) due to antagonistic effects between amino acids. Therefore, NCG supplementation represents a more feasible approach. As a metabolically stable activator of CPS-I, NCG promotes arginine and citrulline synthesis in porcine intestinal cells. This study found that NCG supplementation promoted arginine synthesis in suckling kidlets, significantly increasing plasma free arginine, citrulline and ornithine contents without reducing plasma tryptophan and lysine contents, indicating that NCG promotes endogenous arginine synthesis without affecting lysine and ornithine transport and absorption.

Arginine is an important immune regulator. Studies have shown that dietary arginine supplementation significantly increases immune organ development (spleen and bursa of Fabricius) in chickens and geese, and affects thymus index in meat rabbits. This study found that NCG supplementation significantly increased spleen relative weight in suckling kidlets, consistent with previous results and suggesting that NCG may promote immune organ development and immune regulation. In monogastric animals, arginine regulates the mammalian target of rapamycin (mTOR) signaling pathway in intestinal epithelial cells, promoting intestinal protein synthesis, inhibiting protein degradation, and stimulating proliferation and growth of intestinal mucosal epithelial cells. Dietary arginine supplementation significantly increased small intestine relative weight, villus height and crypt depth in 21-day-old weaned piglets, promoting intestinal development. Recent studies have also shown that dietary supplementation with rumen-protected arginine and NCG increased intestinal mucosal protein synthesis rate in Xinjiang fine-wool weaned lambs. This study demonstrated that NCG supplementation significantly increased large and small intestine relative weights, promoting intestinal development. As the primary site for nutrient digestion and absorption, enhanced intestinal growth can improve nutrient utilization, which may explain the improved growth performance observed in NCG-supplemented kidlets.

In conclusion, oral NCG supplementation at appropriate doses effectively increases plasma arginine and its metabolite levels, reduces diarrhea rate, improves growth performance and promotes organ development in suckling kidlets.

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