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Ethics approval and consent to participate	The animal care and experimental protocols used in the present study were approved by the Institution of Animal Care and Use Committee, Kangwon National University. (Ethical code: KW-240722-1).

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Abstract

Reducing dietary crude protein (CP) levels while supplementing with essential amino acids (AA) has been widely studied as a strategy to maintain nutrient balance while lowering nitrogen excretion. Among feed additives, N-carbamylglutamate (NCG), an arginine precursor with superior stability and mitochondrial permeability, has emerged as a promising feed additive for improving intestinal development and overall pig performance. A total of 240 crossbred grower pigs were allotted to four dietary treatments based on initial body weight. Treatments were arranged in a 2 × 2 factorial design with two levels of dietary CP (MCP, 16% moderate CP; LCP, 14% low CP) and two levels of NCG supplementation (WN, without NCG; NS, 0.1% NCG supplementation). Each treatment had 10 replicates, with six (3 male and 3 female) pigs per pen, and the feeding trial lasted for 4 weeks. Pigs fed the MCP diet tended to have higher final BW ($p = 0.088$) compared with the LCP diet. Feed efficiency was greater in MCP than LCP ($p < 0.05$) and also tended to be enhanced in the NS ($p = 0.089$). CP digestibility tended to increase in the NS group compared with the WN ($p = 0.093$). The digestibility of arginine and tyrosine was increased in the NS group compared with the WN group ($p < 0.05$). Furthermore, lysine ($p = 0.067$), glycine ($p = 0.058$), and the average of essential AA ($p = 0.091$) digestibility tended to be increased in the NS. The NS group showed higher concentrations of arginine, lysine, essential AA, non-essential AA, and total AA in the blood ($p < 0.05$). Trypsin activity showed a tendency to increase in the NS group compared with the WN group ($p = 0.087$). In conclusion, dietary NCG supplementation in a low-protein diet showed potential to improve feed efficiency, AA digestibility, and serum AA profiles in early growing pigs.

Keywords: Growth performance, arginine, nitric oxide, digestibility, amino acid.

Introduction

In recent years, environmental regulations on the swine industry have become increasingly stringent due to the intensifying impacts of climate change and environmental pollution [1,2]. Among the various factors contributing to the environmental footprint of pig production, nitrogen excretion has received considerable attention because of its adverse effects, including water pollution, acidification, and greenhouse gas emissions [3]. Moreover, excessive dietary nitrogen intake through high crude protein (CP) levels not only reduces feed efficiency but also disrupts intestinal microbiota balance, increases odor emissions, and elevates the risk of diarrhea [4]. To address these issues, numerous studies have focused on reducing dietary CP levels by supplementing essential amino acid (AA), and maintaining nutrient balance while minimizing nitrogen excretion [5,6]. In particular, arginine supplementation has gained attention for its potential to optimize AA profiles, support intestinal development, and enhance immune function [7,8]. However, the practical effects of arginine supplementation on growth performance remain limited. This is primarily due to its short half-life, competitive interactions with other AA such as lysine, tryptophan, and histidine, and its susceptibility to degradation by arginase. Additionally, arginine is highly degraded by intestinal microbial metabolism [9], which may further compromise its bioavailability. Therefore, further research is warranted to overcome the limitations of arginine supplementation and to develop strategies that effectively incorporate arginine into low-CP diets without compromising pig health or performance. N-carbamylglutamate (NCG) has recently attracted considerable attention as a promising feed additive due to its role as a precursor of arginine [10,11]. Structurally, NCG is analogous to N-acetylglutamate, which serves as a critical cofactor for carbamoyl phosphate synthetase I as an essential enzyme in the urea cycle [12]. In addition to its functional similarity, NCG exhibits favorable physicochemical properties, including high mitochondrial permeability and a longer half-life compared to arginine, making it suitable for nutritional applications [9,13]. These characteristics enable NCG to enhance endogenous arginine synthesis, which in turn promotes the production of important antioxidant metabolites such as nitric oxide (NO) and polyamines [14]. The greater systemic antioxidant capacity may support the growth performance of the recent fast-growing pigs in high stocking density in commercial farms. Therefore, it was reported that dietary supplementation with NCG has been associated with reduced ammonia emissions and improved nitrogen utilization [15,16]. In weanling pigs, NCG has been shown to alleviate weaning stress by enhancing growth performance and supporting intestinal development and health [17,18]. Furthermore, in growing-finishing pigs, NCG supplementation has been linked to improvements in carcass quality and meat characteristics.

Despite these promising outcomes, the effects of NCG in early growing pigs remain relatively underexplored. Therefore, the present study was conducted to evaluate the efficacy of NCG supplementation on growth performance, nutrient digestibility, blood AA profiles, and intestinal enzyme activities in early growing pigs.

Materials and Methods

Ethical Statement

The Animal Care and Use Committee of Kangwon National University (Chuncheon, South Korea) examined and approved the experimental procedure employed in this study (KW-240722-1).

Animals and Experimental Design

The experiment was conducted on a commercial pig farm in Haman, Korea. Growing pigs with an average initial body weight (BW) of 20.38 ± 2.65 kg of the Landrace × Yorkshire × Duroc breeds were housed in partially slatted concrete floor pens equipped with feeders and nipple drinkers. Feed and water were given as *ad-libitum*. Pigs were placed in a pen of 2.80 m × 5.00 m diameter. The experimental environment was controlled at 24 ℃ of temperature and 70 % of relative humidity by an automatic environment control system. A total of 240 crossbred grower pigs were allotted based on their initial BW in four treatments. Treatments were divided by dietary CP level (MCP, 16% of moderate CP in diet; LCP, 14% of low CP in diet) and NCG supplementation (WN, without NCG; NS, 0.1% NCG supplementation). NCG (≥97% purity) was sourced from ANIMORE SCI and TECH Co., Ltd. (Beijing, China). Each treatment had 10 replicates, with six pigs (3 males and 3 females) per replicate, over a 4-week period. The experimental diet was formulated to meet the nutritional requirements of pigs recommended by NRC (2012) (Table 1).

Growth Performance

The experiment assessed growth performance at the start and end of each phase by examining data related to average daily gain (ADG), average daily feed intake (ADFI), BW, and feed efficiency (G:F). The G:F was determined through the computation of the ADG divided by the ADFI. Throughout the entire experiment, mortality was monitored, but no instances were recorded.

Sample collection and chemical analysis

Digestibility of dry matter (DM), CP, gross energy (GE), and ether extract (EE) was determined using chromium oxide (Cr_2O_3) as markers. For 7 days prior to the end of the experiment, growing pigs were fed powder feed mixed with 2.5 g/kg Cr_2O_3. Fecal samples were collected on day 3 before the end of the trial and mixed in each pen to obtain a homogeneous representative sample; then a subsample was taken and frozen at -20 ℃ for further examination. Feed and fecal samples were analyzed for nutrient digestibility. Fecal samples were thawed and dried for 72 h in an Advantec FC-610 oven (Toyo Seisakusho) operating at 75 °C for the evaluation of DM content. Before proximate analysis, the dried fecal samples were first ground to a suitable size through a 1-mm filter (Christy and Norris Hammer Mill). Estimation of CP content was made using method 990.03 (AOAC, 2016). A Soxhlet extraction (Soxtec 2050, FOSS North America) was used for EE analysis using method 954.02 (AOAC, 2016). A bomb calorimeter (Parr 1261 bomb calorimeter, Parr Instruments) was used to evaluate GE content.

At the end of this experiment, pigs were euthanized to take samples for apparent ileal digestibility (AID) of AA. The digesta samples were taken from the distal part of the ileum and stored in the refrigerator at -20 ℃ until the analysis. The digesta samples underwent a thawing process prior to freeze-drying. The determination of AA was done by hydrolyzing samples in glass tubes sealed under vacuum for 24 h with 6 N HCl (including phenol) at 110 ± 2 °C. Methionine and cysteine analysis were determined after oxidation with performic acid. The identification of AA was conducted using a Waters ion-exchange High-Performance Liquid Chromatography system, and the software (version 3.05.01, Millennium, Waters, Millipore) was used to integrate the chromatograms following the method established by Hosseindoust et al. [19].

$$\text{Nutrient digestibility (\%)} = [1 - (N_{digesta} / N_{diet}) \times (Cr_{diet} / Cr_{digesta})] \times 100,$$

In the equations provided,

N_{diet} and $N_{digesta}$ represents the nutrient concentrations of the diet and digesta, respectively (% of DM); Cr_{diet} and $Cr_{digesta}$ are the Cr_2O_3 of the diet and output, respectively (% of DM).

Digesta enzyme activity

Digesta enzyme activity of trypsin, chymotrypsin, protease, amylase, and lipase activity was analyzed from all pigs following the assay kit instructions. Trypsin (Abcam Limited, Cambridge, England catalog No. ab102531), Chymotrypsin (Abcam Limited, Cambridge, England catalog No. ab234051), Protease (Abcam Limited, Cambridge, England catalog No. ab111750.), Amylase (Abcam Limited, Cambridge, England catalog No. ab102523), and Lipase (Abcam Limited, Cambridge, England catalog No. ab102524).

Blood Amino Acid Profile and Urea Nitrogen

Blood samples (10 mL each) were collected from two selected pigs per pen from the middle range of BW in the group. Sampling was performed via vena cava venipuncture using disposable Vacutainer SST II Plus plastic serum tube (367955, Becton Dickinson, Franklin, NJ, USA). The serum was then separated from the blood samples by centrifuging them for 15 minutes at 4 °C at 3,000 g. After that, the blood sample was stored at -20 °C until analysis. Blood urea nitrogen (BUN) levels were determined using a Cobas 6000 analyzer (Roche Diagnostics, Basel, Switzerland). The analysis was performed using the kinetic/potentiometric method, which measures the rate of change in absorbance at a specific wavelength to quantify the concentration of BUN in the serum samples.

Statistical Analysis

Data generated in the present study were statistically analyzed by adopting two-way ANOVA using the GLM procedure by the SAS (SAS Institute, Cary, NC, USA). Factorial arrangement was analyzed by dietary CP level and NCG supplementation. Significant differences between treatment means were identified using Tukey's honest significant difference test. Data were expressed as means and standard deviations, with values of $p < 0.05$ classified as statistically significant, and values of $0.05 \leq p < 0.1$ were considered as a tendency.

Results

Growth Performance

Pigs fed the MCP diet tended to have a higher final BW compared to those on the LCP diet ($p = 0.088$), although the difference was not statistically significant. NCG supplementation did not significantly affect final BW (Table 2). The ADG was greater in the MCP group than in the LCP group ($p < 0.05$). Similarly, the G:F was significantly

improved in pigs fed the MCP diet ($p < 0.05$), and showed a trend towards improvement with NCG supplementation ($p = 0.089$). The ADFI was not influenced by either dietary CP level or NCG supplementation. No significant interaction between dietary CP level and NCG supplementation was observed for any growth performance parameter.

Nutrient Digestibility

The CP digestibility tended to be higher in pigs receiving NCG supplementation (NS) compared to those without NCG (WN) ($p = 0.093$; Table 3). However, the digestibility of DM, GE, and EE was unaffected by NCG supplementation. The dietary CP level had no significant effect on nutrient digestibility.

Apparent Ileal Amino Acid Digestibility

The AID of arginine and tyrosine was significantly greater in the NS group than in the WN group ($p < 0.05$; Table 4). Additionally, AID of lysine ($p = 0.067$), glycine ($p = 0.058$), and the average of essential AA ($p = 0.091$) showed trends towards improvement with NCG supplementation. The AID of serine and the average of non-essential AA exhibited significant interaction effects between dietary CP level and NCG supplementation ($p < 0.05$).

Enzyme Activity

Trypsin activity in the jejunal digesta tended to increase in the NS group compared to the WN group ($p = 0.087$; Table 5). However, the activities of chymotrypsin, protease, amylase, and lipase were not significantly affected by dietary CP level or NCG supplementation.

Blood Profile

The LCP diet significantly reduced lysine and alanine concentrations in the blood compared to the MCP group ($p < 0.05$; Table 6). Furthermore, BUN ($p = 0.073$), arginine ($p = 0.069$), valine ($p = 0.053$), and the average essential AA concentration ($p = 0.063$) tended to be lower in the LCP group. The NS group exhibited significantly higher concentrations of arginine, lysine, essential AA, non-essential AA, and total AA in the blood ($p < 0.05$). Significant interaction effects between dietary CP level and NCG supplementation were observed for arginine and essential AA concentrations ($p < 0.05$).

Discussion

The results of the present study demonstrated a beneficial trend of NS in enhancing feed efficiency when incorporated into the LCP diet for early growing pigs. The early growing period is a critical phase that demands rapid muscle accretion to ensure optimal transition into the finishing stage [6,20]. However, LCP diets, although effective in reducing nitrogen excretion and odor emissions, may fail to supply sufficient protein and AA to support ideal growth and muscle development [21]. NCG, a functional analog and metabolic precursor of arginine, plays a pivotal role in promoting muscle protein synthesis [10]. NCG also serves as a precursor for mammalian target of rapamycin (mTOR), a protein kinase that stimulates muscle protein synthesis and reduces BUN levels [22,23]. Additionally, NCG-derived NO has been shown to enhance glucose uptake and lipolysis and support energy metabolism and overall growth [24]. In line with our findings, Li et al. [22] reported that supplementation with 0.08% NCG in finishing pigs improved ADG even when dietary CP was reduced by 2.4%. Similarly, Hu et al. [18] observed that 0.05% NCG supplementation in weaning pigs enhanced growth performance during the experimental period. Collectively, these results suggest that NCG supplementation in LCP diets may enhance crude protein utilization and support muscle synthesis, ultimately contributing to improved feed efficiency in early growing pigs.

In the present study, no significant differences were observed in the apparent digestibility of DM, GE, and EE among dietary treatments. However, CP digestibility tended to be higher in the NS group compared to the WN group. Nutrient digestibility in pigs is influenced by multiple physiological factors, including the composition of the intestinal microbiota, gut morphology, expression of tight junction proteins, and digestive enzyme activity [26]. NCG has been reported to enhance intestinal development by promoting the epithelial cell function and maintaining structural integrity [27]. Furthermore, it has been shown to increase villus height and upregulate the expression of tight junction proteins, thereby supporting efficient nutrient absorption [18]. These effects are likely associated with NCG's role to enhance antioxidant capacity via NO production, particularly by stimulating the development and function of intestinal mitochondria [28]. Our study also suggests that NCG supplementation improves nutrient digestibility through the enhancement of intestinal health and development. The AID of arginine and lysine was greater in the NS group compared to the WN group. Both arginine and lysine are primarily absorbed in the jejunum via AA transporters, particularly members of the solute-carrier family [29]. NCG supplementation is known to enhance the expression of these AA transporters by stimulating the production of

196 goblet cells and mucin secretion, thereby strengthening the intestinal mucosal barrier [18,30]. Wang et al. [27]
197 demonstrated that the inclusion of NCG in a diet with 6% reduced crude protein significantly improved the AID
198 and bioavailability of AA in growing pigs. In our study, jejunal trypsin activity was elevated in the NS group.
199 Trypsin, a key proteolytic enzyme involved in the digestion of arginine and lysine, is activated by enterokinase,
200 an enzyme secreted by the duodenal mucosa [31]. The observed increase in trypsin activity may be attributed to
201 the stimulatory effect of NCG on intestinal mucosal proliferation, possibly mediated through activation of the
202 mTOR pathway and subsequent protein synthesis within intestinal epithelial cells [32]. NCG supplementation
203 may indirectly supports intestinal barrier function while activating the antioxidant status in gut mitochondria.

204 In this study, BUN levels decreased as dietary CP levels were reduced. However, NCG supplementation did not
205 exert an additional effect on this reduction. BUN is a metabolic by-product of protein degradation and serves as a
206 reliable indicator of protein metabolism and nitrogen utilization efficiency in pigs [33]. NCG has been reported
207 to reduce BUN levels by enhancing nitrogen metabolism and facilitating more efficient excretion of nitrogenous
208 waste. Wang et al. [32] observed that BUN concentrations were significantly reduced with NCG supplementation
209 when pigs were fed diets with identical CP levels. In contrast, Li et al. [25] reported that NCG supplementation
210 in low-CP diets did not affect BUN concentrations compared to low-CP diets without NCG. These findings
211 suggest that the effect of NCG on BUN concentration may be influenced by the overall dietary protein and AA
212 content. In addition to its potential effect on nitrogen excretion, NCG supplementation in the current study was
213 associated with increased serum concentrations of arginine, lysine, and isoleucine. The measurement of serum
214 AA levels may provide a more sensitive indicator of the metabolic efficacy of NCG than BUN alone. Compared
215 to arginine, NCG possesses a longer half-life, which may contribute to sustained stimulation of mitochondrial
216 function and activation of the mTOR signaling pathway, which are critical regulators of protein synthesis [9,13].
217 Consistent with our findings, Wang et al. [34] also reported that NCG supplementation increased circulating AA
218 levels in growing pigs, further supporting the notion that NCG can enhance systemic AA availability and
219 utilization. Taken together, these results suggest that NCG may play a beneficial role in optimizing protein
220 metabolism and AA efficiency, particularly under conditions of reduced dietary CP.

221

Conclusion

The findings of this study suggest that NS supplementation in LCP diets tended to improve feed efficiency in early growing pigs. NCG also tended to enhance crude protein digestibility and significantly increased the apparent ileal digestibility of arginine and lysine. These effects may be attributed to the stimulatory role of NCG on circulating amino acid concentrations. Overall, the results highlight the potential of NCG to improve growth-related physiological parameters, particularly under protein-restricted dietary conditions.

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Declaration of interest statement

The authors report there are no competing interests to declare.

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Tables

Table 1. Ingredients and calculated composition of diet (as-fed).

Item	MCP	LCP
Ingredients, %		
Corn	64.07	67.98
Soybean meal	20.33	16.58
DDGS	8.00	8.00
Animal fat	2.13	2.08
Molasses	3.00	3.00
L-lysine (78%)	0.24	0.18
Limestone	0.98	0.96
Dicalcium phosphate	0.55	0.52
Salt	0.30	0.30
Vitamin premix[1]	0.15	0.15
Mineral premix[2]	0.15	0.15
Choline chloride	0.05	0.05
Phytase	0.05	0.05
Total	100	100
Chemical composition, %		
ME, kcal/kg	3,300	3,300
Crude protein	16.00	14.00
Crude fat	4.85	4.79
Calcium	0.66	0.66
Available phosphorus	0.31	0.31
SID lysine	0.98	0.86
SID methionine+cysteine	0.55	0.48
SID threonine	0.59	0.52
SID tryptophan	0.17	0.15
Analyzed composition, %		
Gross energy, kcal/kg	4,615	4,626
Crude protein	16.32	14.20
Calcium	0.70	0.69
Phosphorus	0.57	0.56
Lysine	1.15	0.98
Methionine	0.33	0.28
Threonine	0.73	0.66
Tryptophan	0.20	0.18

MCP, moderate crude protein; LCP, low protein level; DDGS, dried distiller's grains with solubles; Me, metabolizable energy; SID, standardized ileal digestibility.

[1]Supplied per kg of diet: 16,000 IU vitamin A (palmitate), 2.00 mg vitaminB_1 (thiamin), 5.00 mg vitamin B_2 (riboflavin), 2.00 mg vitamin B_6 (pyridoxine), 0.03 mg vitamin B_{12} (cyanocobalamin), 25.00 mg niacin, 0.40 mg folic acid, 0.05 mg biotin, 5.00 mg ethoxyquin, 2,000 IU vitamin D_3 (cholecalciferol), 75.00 mg vitamin E (dl-α-tocopheryl acetate), 2.00 mg vitamin K_3 (menadione).

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Table 2. Effects of diet protein level and N-carbamylglutamate (NCG) supplementation on growth performance in growing pigs.

Diet (D)	MCP		LCP		SEM	P-value[3]		
NCG (N)[1]	-	+	-	+		D	N	D × N
Initial BW, kg	20.31	20.45	20.36	20.41	0.247	0.998	0.847	0.926
Final BW, kg	39.46	40.05	38.69	39.47	0.234	0.088	0.148	0.911
ADG, kg/d	0.684	0.700	0.655	0.681	0.012	0.023	0.107	0.980
ADFI, kg/d	1.324	1.322	1.327	1.325	0.048	0.531	0.647	0.779
G:F	0.517	0.530	0.494	0.514	0.012	0.023	0.089	0.973

MCP, moderate 16% crude protein level; LCP, low 14% crude protein level; SEM, standard error of means; BW, body weight; ADG, average daily weight gain; ADFI, average daily feed intake; G:F, feed efficiency.

[1]0.1 % NCG supplementation.

Table 3. Effects of diet protein level and N-carbamylglutamate (NCG) supplementation on nutrient digestibility in growing pigs.

Diet (D)	MCP		LCP		SEM	P-value[3]		
NCG (N)[1]	-	+	-	+		D	N	D×N
DM	85.09	84.97	85.04	85.48	1.138	0.425	0.567	0.320
GE	76.05	76.18	76.36	76.15	1.231	0.470	0.928	0.716
CP	79.89	81.29	79.71	81.99	1.129	0.364	0.093	0.805
EE	43.06	43.01	43.06	42.73	1.108	0.511	0.381	0.872

MCP, moderate 16% crude protein level; LCP, low 14% crude protein level; SEM, standard error of means; DM, dry matter; GE, gross energy; CP, crude protein; EE, ether extract.
[1]0.1 % NCG supplementation.

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Table 4. Effects of diet protein level and N-carbamylglutamate (NCG) supplementation on apparent ileal amino acid digestibility in growing pigs.

Diet (D)	MCP		LCP		SEM	P-value[3]		
NCG (N)[1]	-	+	-	+		D	N	D × N
Essential amino acid								
Arginine	86.73	87.77	86.93	88.35	0.75	0.459	0.025	0.728
Histidine	89.43	88.91	88.45	88.39	0.85	0.226	0.638	0.699
Isoleucine	85.59	85.99	85.79	85.89	0.95	0.940	0.712	0.824
Leucine	87.02	86.10	85.78	85.73	0.76	0.147	0.377	0.421
Lysine	88.43	89.70	88.31	89.59	0.65	0.062	0.067	0.996
Methionine	91.82	92.07	90.48	91.79	0.84	0.181	0.197	0.383
Phenylalanine	85.99	86.31	86.69	86.32	0.88	0.570	0.974	0.584
Threonine	83.24	82.89	82.55	83.45	0.76	0.905	0.613	0.255
Tryptophan	93.45	92.67	91.73	92.87	0.79	0.182	0.748	0.094
Valine	87.52	87.79	86.62	88.03	0.93	0.622	0.212	0.394
Non-essential amino acid								
Alanine	88.36	88.40	88.21	88.37	0.86	0.878	0.875	0.922
Aspartic acid	81.07	82.33	81.89	80.88	0.97	0.656	0.859	0.108
Cystine	69.89	69.44	71.25	69.54	0.92	0.275	0.105	0.338
Glutamic acid	88.81	88.20	88.28	87.99	1.04	0.621	0.545	0.826
Glycine	86.19	88.16	86.89	87.27	0.84	0.874	0.058	0.191
Proline	85.09	85.76	84.96	85.21	0.92	0.606	0.491	0.754
Serine	85.27	86.61	85.90	84.01	0.80	0.092	0.632	0.007
Tyrosine	84.84	86.12	84.65	86.44	0.91	0.920	0.023	0.689
Average								
Essential	87.93	88.03	87.34	88.05	0.33	0.232	0.091	0.199
Non-essential	83.70	84.38	84.01	83.72	0.31	0.423	0.371	0.031
Total	86.05	86.41	85.86	86.12	0.31	0.109	0.136	0.742

MCP, moderate 16% crude protein level; LCP, low 14% crude protein level; SEM, standard error of means.

[1]0.1 % NCG supplementation.

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Table 5. Effects of diet protein level and N-carbamylglutamate (NCG) supplementation on jejunal enzyme activity in growing pigs.

Diet (D)	MCP		LCP		SEM	P-value		
NCG (N)[1]	-	+	-	+		D	N	D × N
Trypsin	65.55	68.34	65.81	67.54	1.82	0.836	0.087	0.687
Chymotrypsin	10.28	9.94	10.50	10.86	0.63	0.206	0.987	0.443
Protease	40.07	40.18	39.15	39.39	1.20	0.318	0.840	0.937
Amylase	49.34	50.67	49.60	50.09	1.11	0.841	0.256	0.599
Lipase	2.63	2.57	2.42	2.39	0.29	0.348	0.837	0.918

MCP, moderate 16% crude protein level; LCP, low 14% crude protein level; SEM, standard error of means.

[1]0.1 % NCG supplementation.

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Table 6. Effects of diet protein level and N-carbamylglutamate (NCG) supplementation on blood profile in growing pigs.

Diet (D)	MCP		LCP		SEM	P-values		
NCG (N)[1]	-	+	-	+		D	N	D × N
BUN, mmol/L	2.09	1.93	1.89	1.54	0.22	0.073	0.114	0.550
Essential amino acid, nmol/ml								
Arginine	219	232	210	263	8.32	0.069	<0.001	0.001
Histidine	54	56	47	49	2.61	0.271	0.281	0.980
Isoleucine	89	93	81	84	2.32	0.463	0.033	0.982
Leucine	216	218	198	206	6.13	0.188	0.253	0.474
Lysine	321	334	279	302	6.87	0.020	0.001	0.321
Methionine	55	55	43	42	4.68	0.588	0.899	0.904
Phenylalanine	72	71	61	64	3.48	0.225	0.864	0.402
Threonine	169	175	143	153	5.42	0.349	0.136	0.514
Tryptophan	49	50	42	44	3.16	0.714	0.580	0.761
Valine	269	287	228	247	7.78	0.053	0.102	0.907
Non-essential amino acid, nmol/ml								
Alanine	472	468	434	432	4.71	0.019	0.390	0.726
Aspartic acid	22	20	15	14	1.46	0.833	0.287	0.652
Glutamic acid	219	234	184	206	7.26	0.208	0.101	0.496
Glycine	990	1,003	1,008	998	12.99	0.455	0.881	0.222
Proline	270	285	224	245	10.58	0.291	0.222	0.707
Serine	185	192	184	189	8.40	0.715	0.341	0.874
Tyrosine	102	100	101	101	5.82	0.923	0.832	0.798
Average								
Essential	152	157	146	158	3.12	0.063	0.071	0.029
Non-essential	323	329	323	329	3.15	0.883	0.018	0.834
Total	222	228	219	228	5.50	0.216	0.201	0.221

MCP, moderate 16% crude protein level; LCP, low 14% crude protein level; SEM, standard error of means; BUN, blood urea nitrogen.
[1]0.1 % NCG supplementation.

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