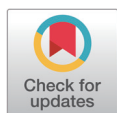


Exploratory assessment of N-carbamylglutamate supplementation on feed efficiency and protein utilization in early growing pigs fed low crude protein diet

So Dam Choi, Jun Young Mun, Abdolreza Hosseindoust, Habeeb Tajudeen, Seon Ah Park, Priscilla Neves Silvestre, Sang Sik Lee, Jin Soo Kim*

Department of Animal Industry Convergence, Kangwon National University, Chuncheon, Korea



Received: May 13, 2025

Revised: Jul 1, 2025

Accepted: Jul 12, 2025

*Corresponding author

Jin Soo Kim

E-mail: kjs896@kangwon.ac.kr

Copyright © 2026 Korean Society of Animal Science and Technology. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID

So Dam Choi

<https://orcid.org/0009-0009-2711-7106>

Jun Young Mun

<https://orcid.org/0000-0002-3075-7157>

Abdolreza Hosseindoust

<https://orcid.org/0000-0001-9191-0613>

Habeeb Tajudeen

<https://orcid.org/0000-0002-5623-3175>

Seon Ah Park

<https://orcid.org/0009-0007-6584-2777>

Priscilla Neves Silvestre

<https://orcid.org/0009-0007-0473-2603>

Sang Sik Lee

<https://orcid.org/0009-0003-2171-8388>

Jin Soo Kim

<https://orcid.org/0000-0002-9518-7917>

Competing interests

No potential conflict of interest relevant to this article was reported.

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 (without NCG [WN]; 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

Funding sources

Not applicable.

Acknowledgements

Not applicable.

Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Kim JS.

Data curation: Hosseindoust A, Neves Silvestre P.

Formal analysis: Choi SD.

Methodology: Choi SD, Mun JY.

Software: Tajudeen H, Neves Silvestre P, Lee SS.

Validation: Mun JY, Hosseindoust A.

Investigation: Mun JY, Park SA.

Writing - original draft: Choi SD.

Writing - review & editing: Choi SD, Mun JY, Hosseindoust A, Tajudeen H, Park SA, Neves Silvestre P, Lee SS, Kim JS.

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).

Declaration of generative AI

No AI tools were used in this article.

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

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 $\times$ Yorkshire $\times$ 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 2.80×5.00 m diameter. The experimental environment was controlled at 24°C 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 (16% of

moderate CP [MCP] in diet; 14% of low CP [LCP] in diet) and NCG supplementation (without NCG [WN]; 0.1% NCG supplementation [NS]). NCG ($\geq 97\%$ purity) was sourced from Beijing Animore Sci & Tech. 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 [19] (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°C 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 [20]. A Soxhlet extraction (Soxtec 2050, FOSS North America) was used for EE analysis using method 954.02 [20]. 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°C 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 6N HCl (including phenol) at $110 \pm 2^\circ\text{C}$. 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, and Millipore) was used to integrate the chromatograms following the method established by Hosseindoust et al. [21].

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

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, catalog No. ab102531), Chymotrypsin (Abcam Limited, catalog No. ab234051), Protease (Abcam Limited, catalog No. ab111750), Amylase (Abcam Limited, catalog No. ab102523), and Lipase (Abcam Limited, catalog No. ab102524).

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

¹⁾Supplied per kg of diet: 16,000 IU vitamin A (palmitate), 2.00 mg vitamin B₁ (thiamin), 5.00 mg vitamin B₂ (riboflavin), 2.00 mg vitamin B₆ (pyridoxine), 0.03 mg vitamin B₁₂ (cyanocobalamin), 25.00 mg niacin, 0.40 mg folic acid, 0.05 mg biotin, 5.00 mg ethoxyquin, 2,000 IU vitamin D₃ (cholecalciferol), 75.00 mg vitamin E (dl- α -tocopheryl acetate), 2.00 mg vitamin K₃ (menadione).

²⁾Supplied per kilogram of diet: 100 mg Fe, 10 mg Cu, 5 mg Mn, 100 mg Zn, 0.2 mg I, 0.4 mg Se.

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

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). 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). 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). 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

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

Diet (D) NCG (N) ¹⁾	MCP		LCP		SEM	p-value		
	–	+	–	+		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

¹⁾0.1 % NCG supplementation.

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.

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

Diet (D) NCG (N) ¹⁾	MCP		LCP		SEM	p-value		
	–	+	–	+		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

¹⁾0.1 % NCG supplementation.

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.

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

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		
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

¹⁾0.1 % NCG supplementation.

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

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 [6,22]. 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 [23]. 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 [24,25]. Additionally, NCG-derived NO has been shown to enhance glucose uptake and lipolysis and support energy metabolism and overall growth [26]. In line with our findings, Li et al. [24] 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 [27,28]. NCG has been reported to enhance intestinal development by promoting the epithelial cell function and maintaining

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

Diet (D) NCG (N) ¹⁾	MCP		LCP		SEM	p-value		
	–	+	–	+		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

¹⁾0.1 % NCG supplementation.

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

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

¹⁾0.1 % NCG supplementation.

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

structural integrity [16]. 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 [29]. 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 [15]. NCG supplementation is known to enhance the expression of these AA transporters by stimulating the production of goblet cells and mucin secretion, thereby strengthening the intestinal mucosal barrier [18,30]. Wang et al. [16] demonstrated that the inclusion of NCG in a diet with 6% reduced crude protein significantly improved the AID and bioavailability of AA in growing pigs. In our study, jejunal trypsin activity was elevated in the NS group. Trypsin, a key proteolytic enzyme involved in the digestion of arginine and lysine, is activated by enterokinase, an enzyme secreted by the duodenal mucosa [31]. The observed increase in trypsin activity may be attributed to the stimulatory effect of NCG on intestinal mucosal proliferation, possibly mediated through activation of the mTOR pathway and subsequent protein synthesis within intestinal epithelial cells [32]. NCG supplementation

may indirectly supports intestinal barrier function while activating the antioxidant status in gut mitochondria.

In this study, BUN levels decreased as dietary CP levels were reduced. However, NCG supplementation did not exert an additional effect on this reduction. BUN is a metabolic by-product of protein degradation and serves as a reliable indicator of protein metabolism and nitrogen utilization efficiency in pigs [33]. NCG has been reported to reduce BUN levels by enhancing nitrogen metabolism and facilitating more efficient excretion of nitrogenous waste. Wang et al. [34] observed that BUN concentrations were significantly reduced with NCG supplementation when pigs were fed diets with identical CP levels. In contrast, Li et al. [27] reported that NCG supplementation in low-CP diets did not affect BUN concentrations compared to low-CP diets without NCG. These findings suggest that the effect of NCG on BUN concentration may be influenced by the overall dietary protein and AA content. In addition to its potential effect on nitrogen excretion, NCG supplementation in the current study was associated with increased serum concentrations of arginine, lysine, and isoleucine. The measurement of serum AA levels may provide a more sensitive indicator of the metabolic efficacy of NCG than BUN alone. Compared to arginine, NCG possesses a longer half-life, which may contribute to sustained stimulation of mitochondrial function and activation of the mTOR signaling pathway, which are critical regulators of protein synthesis [9,13]. Consistent with our findings, Wang et al. [34] also reported that NCG supplementation increased circulating AA levels in growing pigs, further supporting the notion that NCG can enhance systemic AA availability and utilization. Taken together, these results suggest that NCG may play a beneficial role in optimizing protein metabolism and AA efficiency, particularly under conditions of reduced dietary CP.

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.

REFERENCES

1. Rauw WM, Rydhmer L, Kyriazakis I, Øverland M, Gilbert H, Dekkers JCM, et al. Prospects for sustainability of pig production in relation to climate change and novel feed resources. *J Sci Food Agric*. 2020;100:3575–86. <https://doi.org/10.1002/jsfa.10338>
2. Qian Y, Song K, Hu T, Ying T. Environmental status of livestock and poultry sectors in China under current transformation stage. *Sci Total Environ*. 2018;622–623:702–9. <https://doi.org/10.1016/j.scitotenv.2017.12.045>
3. Cappelaere L, Le Cour Grandmaison J, Martin N, Lambert W. Amino acid supplementation to reduce environmental impacts of broiler and pig production: a review. *Front Vet Sci*. 2021;8:689259. <https://doi.org/10.3389/fvets.2021.689259>
4. Limbach JR, Espinosa CD, Perez-Calvo E, Stein HH. Effect of dietary crude protein level on growth performance, blood characteristics, and indicators of intestinal health in weanling pigs. *J Anim Sci*. 2021;99:skab166. <https://doi.org/10.1093/jas/skab166>
5. Liu R, He J, Ji X, Zheng W, Yao W. A moderate reduction of dietary crude protein provide

- comparable growth performance and improve metabolism via changing intestinal microbiota in Sushan nursery pigs. *Animals*. 2021;11:1166. <https://doi.org/10.3390/ani11041166>
6. Mun J, Tajudeen H, Hosseindoust A, Ha S, Park S, Kim J. A reduction in dietary crude protein with amino acid balance has no negative effects in pigs. *J Anim Sci Technol*. 2024;66:493–503. <https://doi.org/10.5187/jast.2023.e64>
 7. Yoon SY, Sa SJ, Cho ES, Ko HS, Choi JW, Kim JS. Effects of zinc oxide and arginine on the intestinal microbiota and immune status of weaned pigs subjected to high ambient temperature. *Animals*. 2020;10:1537. <https://doi.org/10.3390/ani10091537>
 8. Moturi J, Yoon SY, Hosseindoust A, Ha SH, Tajudeen H, Mun JY, et al. Effects of dietary arginine in ameliorating the deleterious effects induced by mycotoxins on growth, immune system, body organs in growing pigs. *J Anim Sci Technol*. 2022;64:727–39. <https://doi.org/10.5187/jast.2022.e54>
 9. Palencia JYP, Saraiva A, Abreu MLT, Zangeronimo MG, Schinckel AP, Pospissil Garbossa CA. Effectiveness of citrulline and N-carbamoyl glutamate as arginine precursors on reproductive performance in mammals: a systematic review. *PLOS ONE*. 2018;13:e0209569. <https://doi.org/10.1371/journal.pone.0209569>
 10. Galsgaard KD, Pedersen J, Kjeldsen SAS, Winther-Sørensen M, Stojanovska E, Vilstrup H, et al. Glucagon receptor signaling is not required for N-carbamoyl glutamate- and l-citrulline-induced ureagenesis in mice. *Am J Physiol Gastrointest Liver Physiol*. 2020;318:G912–27. <https://doi.org/10.1152/ajpgi.00294.2019>
 11. Kuznetsov AS, Ostrenko KS. The effect of arginine on piglets growth rates, the efficiency of ammonia utilization, and the use of nitrogen from the diet. Ways to eliminate arginine deficiency. *Svinovodstvo (Moskva)* 2020;8:45–7. <https://doi.org/10.37925/0039-713X-2020-8-45-47>
 12. Hodkovicova N, Halas S, Tosnerova K, Stastny K, Svoboda M. The use of functional amino acids in different categories of pigs – a review. *Vet Med*. 2023;68:299–312. <https://doi.org/10.17221/72/2023-VETMED>
 13. Sonaimuthu P, Senkevitch E, Haskins N, Uapinyoying P, McNutt M, Morizono H, et al. Gene delivery corrects N-acetylglutamate synthase deficiency and enables insights in the physiological impact of L-arginine activation of N-acetylglutamate synthase. *Sci Rep*. 2021;11:3580. <https://doi.org/10.1038/s41598-021-82994-8>
 14. Zhang H, Sun H, Peng A, Guo S, Wang M, Looor JJ, et al. N-carbamylglutamate and l-arginine promote intestinal function in suckling lambs with intrauterine growth restriction by regulating antioxidant capacity via a nitric oxide-dependent pathway. *Food Funct*. 2019;10:6374–84. <https://doi.org/10.1039/C9FO01752F>
 15. Yang HS, Fu DZ, Kong XF, Wang WC, Yang XJ, Nyachoti CM, et al. Dietary supplementation with N-carbamylglutamate increases the expression of intestinal amino acid transporters in weaned Huanjiang mini-pig piglets. *J Anim Sci*. 2013;91:2740–8. <https://doi.org/10.2527/jas.2012-5795>
 16. Wang Y, Han S, Zhou J, Li P, Wang G, Yu H, et al. Effects of dietary crude protein level and N-carbamylglutamate supplementation on nutrient digestibility and digestive enzyme activity of jejunum in growing pigs. *J Anim Sci*. 2020;98:skaa088. <https://doi.org/10.1093/jas/skaa088>
 17. Wu X, Ruan Z, Gao Y, Yin Y, Zhou X, Wang L, et al. Dietary supplementation with L-arginine or N-carbamylglutamate enhances intestinal growth and heat shock protein-70 expression in weanling pigs fed a corn- and soybean meal-based diet. *Amino Acids*. 2010;39:831–9. <https://doi.org/10.1007/s00726-010-0538-y>
 18. Hu N, Mao P, Xiong X, Ma Z, Xie Z, Gao M, et al. Effect of N-carbamylglutamate supplementation on growth performance, jejunal morphology, amino acid transporters, and

- antioxidant ability of weaned pigs. *Animals*. 2023;13:3183. <https://doi.org/10.3390/ani13203183>
19. NRC (National Research Council), Division on Earth, Life Studies, Committee on Nutrient Requirements of Swine. Nutrient requirements of swine. 11th rev. ed. The National Academies Press; 2012.
20. AOAC (Association of Official Analytical Chemists). Official methods of analysis of international. 20th ed, Benjamin Franklin Station; 2016.
21. Hosseindoust A, Ha SH, Mun JY, Kim JS. Effects of meal processing of black soldier fly on standardized amino acids digestibility in pigs. *J Anim Sci Technol*. 2023;65:1014-23. <https://doi.org/10.5187/jast.2023.e28>
22. Dong W, Zhang G, Li Z, Liu L, Zhang S, Li D. Effects of different crude protein and dietary fiber levels on the comparative energy and nutrient utilization in sows and growing pigs. *Animals*. 2020;10:495. <https://doi.org/10.3390/ani10030495>
23. Pomar C, Andretta I, Remus A. Feeding strategies to reduce nutrient losses and improve the sustainability of growing pigs. *Front Vet Sci*. 2021;8:742220. <https://doi.org/10.3389/fvets.2021.742220>
24. Li YX, Moreira VR, Wang LS, Liu QL, Gao PD, Zhang XJ, et al. N-carbamylglutamate, a promising functional feed additive in swine production: a review. *Anim Feed Sci Technol*. 2023;303:115719. <https://doi.org/10.1016/j.anifeedsci.2023.115719>
25. Suryawan A, Jeyapalan AS, Orellana RA, Wilson FA, Nguyen HV, Davis TA. Leucine stimulates protein synthesis in skeletal muscle of neonatal pigs by enhancing mTORC1 activation. *Am J Physiol Endocrinol Metab*. 2008;295:E868-75. <https://doi.org/10.1152/ajpendo.90314.2008>
26. Zhang H, Liu X, Ren S, Elsabagh M, Wang M, Wang H. Dietary N-carbamylglutamate or l-arginine supplementation improves hepatic energy status and mitochondrial function and inhibits the AMP-activated protein kinase-peroxisome proliferator-activated receptor γ coactivator-1 α -transcription factor A pathway in intrauterine-growth-retarded suckling lambs. *Anim Nutr*. 2021;7:859-67. <https://doi.org/10.1016/j.aninu.2021.02.005>
27. Li PL, Wu F, Wang YM, Wang CP, Zhou JY, Zeng XF, et al. Combination of non-protein nitrogen and N-carbamylglutamate supplementation improves growth performance, nutrient digestibility and carcass characteristics in finishing pigs fed low protein diets. *Anim Feed Sci Technol*. 2021;273:114753. <https://doi.org/10.1016/j.anifeedsci.2020.114753>
28. Wealleans AL, Bierinckx K, di Benedetto M. Fats and oils in pig nutrition: factors affecting digestion and utilization. *Anim Feed Sci Technol*. 2021;277:114950. <https://doi.org/10.1016/j.anifeedsci.2021.114950>
29. Xing Y, Wu X, Xie C, Xiao D, Zhang B. Meat quality and fatty acid profiles of Chinese Ningxiang pigs following supplementation with N-carbamylglutamate. *Animals*. 2020;10:88. <https://doi.org/10.3390/ani10010088>
30. Duanmu Q, Tan B, Wang J, Huang B, Li J, Kang M, et al. The amino acids sensing and utilization in response to dietary aromatic amino acid supplementation in LPS-induced inflammation piglet model. *Front Nutr*. 2022;8:819835. <https://doi.org/10.3389/fnut.2021.819835>
31. Kurz A, Seifert J. Factors influencing proteolysis and protein utilization in the intestine of pigs: a review. *Animals*. 2021;11:3551. <https://doi.org/10.3390/ani1123551>
32. Wang C, Shang L, Guo Q, Duan Y, Han M, Li F, et al. Effectiveness and safety evaluation of graded levels of N-carbamylglutamate in growing-finishing pigs. *Anim Nutr*. 2022;10:412-8. <https://doi.org/10.1016/j.aninu.2022.04.012>
33. Berghaus D, Haese E, Weishaar R, Sarpong N, Kurz A, Seifert J, et al. Nitrogen and lysine utilization efficiencies, protein turnover, and blood urea concentrations in crossbred grower

- pigs at marginal dietary lysine concentration. *J Anim Sci.* 2023;101:skad335. <https://doi.org/10.1093/jas/skad335>
34. Wang YM, Yu HT, Zhou JY, Zeng XF, Wang G, Cai S, et al. Effects of feeding growing-finishing pigs with low crude protein diets on growth performance, carcass characteristics, meat quality and nutrient digestibility in different areas of China. *Anim Feed Sci Technol.* 2019;256:114256. <https://doi.org/10.1016/j.anifeedsci.2019.114256>