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Abstract

The efficient utilization of dietary protein is a critical factor in modern pig production. This study aimed to evaluate the dose-response effects of dietary N-carbamylglutamate (NCG) supplementation on growth performance, nutrient digestibility, and blood profiles in crossbred grower-finisher pigs fed low-protein diets. One hundred and fifty pigs with an average initial body weight of 50.85 ± 3.11 kg were assigned to five feeding strategies. The treatments consisted of a control diet (NP), and low protein diets (LP) with NCG supplementation (0, 0.4, 0.7, and 1.0 g/kg) 0 over three phases (phase 1, d 1-21; phase 2, d 22-42; phase 3, d 43-63). The data were analyzed using the GLM procedure of SAS. The NCG supplementation linearly improved gain to feed ratio in phases 1 and 2, and overall. The NP group showed a greater gain-to-feed ratio in phases 1 and 3 and a greater overall average daily gain compared to LP treatment. Digestibility of crude protein was linearly increased with supplementation of NCG. Digestibility of Arg, His, Leu, Lys, Phe, Thr, Val, Ala, Glu, Gly, Ser Pro, Cys, Cit, and Orn was linearly improved with NCG supplementation. Digestibility of Arg, His, Ile, Val, Glu, Pro, and Cit was higher in the NC treatment compared with the LP. The gene expression of solute carrier transporter (SLC)6A19, SLC7A5, SLC7A7, SLC7A9, SLC38A2, and mechanistic target of rapamycin in the jejunum was linearly increased with supplementation of NCG. The marketing age and backfat thickness were linearly reduced with supplementation of NCG, and the slaughter weight, carcass weight, and loin muscle area were linearly increased. The NP treatment showed a lower marketing age, and a higher slaughter weight, carcass weight, and dressing percentage compared to the LP. The 0.10% dietary NCG supplementation is recommended for pigs due to linear increase in growth performance, feed efficiency, amino acid utilization, and carcass quality.

Keywords: Arginine, Amino acid, Urea, Absorption, Performance, Transporters

INTRODUCTION

The efficient utilization of dietary protein is a critical factor in modern pig production with direct influence on growth performance, feed costs, and environmental nitrogen emissions [1–3]. Traditionally, high-protein diets have been used to meet the amino acid requirements of rapidly growing pigs; however, excess protein not only increases production costs but also leads to elevated nitrogen excretion and environmental pollution [4–6]. Consequently, there is growing interest in developing strategies that can reduce dietary crude protein (CP) levels without compromising animal performance or nutrient utilization.

N-carbamylglutamate (NCG) is a structural analog of N-acetylglutamate and a precursor of arginine with a promising role as a feed additive [7,8]. Compared with Arg, NCG contains a carbamyl group that confers higher thermo stability, chemical stability, and resistance to hydrolysis by arginase and digestive enzymes in the gastrointestinal tract [9,10]. This structural feature allows NCG to reach target tissues intact and act as a stable analog of N-acetylglutamate, activating carbamoyl phosphate synthetase I in mitochondria [2,11]. Through this sustained activation, NCG continuously stimulates endogenous Arg synthesis and prolongs Arg availability, supporting nitric oxide and polyamine production, antioxidant defense, and protein metabolism [12,13]. NCG can serve as a precursor for nitric oxide synthesis, which plays a key role in vascular function, antioxidant capacity, and cellular homeostasis [14–16]. This effect could be particularly important for modern genetically improved fast-growing pigs, which experience greater physiological stress under commercial stocking density conditions compared with previous generations.

Several studies have demonstrated that dietary NCG supplementation can enhance growth performance, nitrogen retention, and amino acid utilization in pigs [17–19]. These positive effects can be highlighted more under conditions of dietary protein restriction [20]. The underlying mechanisms by which NCG improves performance in low-protein diets involve enhanced amino acid absorption [21,22], increased expression of amino acid transporters [13,23,24], and modulation of signaling pathways related to protein synthesis, such as the mechanistic target of rapamycin (mTOR) [25,26]. Moreover, previous studies have

reported that NCG may improve gut morphology, antioxidant status, and intestinal health in animals [7,8,10]. However, these responses appear to depend on animal species, physiological stage, and dietary conditions, and their relevance under low-protein grower-finisher pig production systems remains unclear. Understanding these effects is essential to determine the optimal inclusion level of NCG that can support sustainable pig production while maintaining or improving performance outcomes.

Therefore, the objective of this study was to evaluate the effects of different levels of NCG supplementation in a low-protein diet on growth performance, nutrient digestibility, blood urea nitrogen concentration, gene expression of amino acid transporters, and carcass traits in pigs.

MATERIALS AND METHODS

Animal and experimental design

A total of 150 growing-finishing pigs (Landrace × Yorkshire × Duroc), with an initial average body weight (BW) of 50.85 ± 3.11 kg, were housed in pens equipped with both partially slatted and solid concrete floors (2.8 × 2.0 m). Each pen was fitted with a nipple drinker and a self-feeder to ensure unrestricted access to water and feed. Pigs were randomly allocated to one of five dietary treatments: a standard protein (NP) diet or a low protein (LP) diet supplemented with NCG at 0, 0.4, 0.7, or 1.0 g/kg. Each treatment group included six replicate pens with five pigs per pen. Diets (Table 1) were formulated to meet or exceed nutrient requirements outlined by the NRC [27]. BW was measured on days 21, 42, and 63. Average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G:F) were calculated at the end of each phase, considering the mortalities. NCG (≥97% purity) was sourced from ANIMORE SCI and TECH Co., Ltd. (Beijing, China).

Nutrient digestibility evaluation

Nutrient digestibility was assessed to determine the apparent digestibility of dry matter (DM), gross energy (GE), CP, and ether extract (EE) at the end of each growth phase. Fecal samples were obtained from two pigs per pen on day 21 (phase 1), day 42 (phase 2), and day 63 (phase 3). These pigs were selected and housed individually for sample collection. Chromium oxide was used as an indigestible marker, incorporated into feed at 2.5 g/kg, two days prior to fecal collection [28]. Feces were collected twice daily at 7:00 a.m. and 3:30 p.m., then pooled per pig, sealed, and stored at −20°C. Samples were subsequently dried at 60°C for 72 h in a forced-air oven and ground using a 1-mm screen (Thomas Model 4 Wiley Mill). The DM and CP analyses were performed using standard AOAC procedures. Cr_2O_3 concentrations were quantified using a spectrophotometer (Jasco V-650) and a bomb calorimeter (Parr 1261).

Serum biochemical analyses

Blood samples were collected from two pigs per pen, selected for having body weights closest to the pen average. Approximately 10 mL of blood was drawn from the vena cava using disposable vacuum tubes (Vacutainer SST II Plus). Samples were centrifuged at 3,000 × g for 15 minutes at 4°C, and the serum was stored at −78°C until analysis. Amino acid profiles were measured using HPLC (Agilent 1260) with a C18 column (3.9 mm × 150 mm × 4 µm) after serum deproteinization. Blood urea nitrogen (BUN) concentrations were determined using a Cobas 6000 analyzer (Roche Diagnostics) via a kinetic/potentiometric method based on changes in absorbance.

Gene expression analysis of amino acid transporters

Jejunal samples were harvested post-mortem from pigs at a commercial abattoir and immediately frozen for RNA extraction (Table 2) using TRI-reagent (Sigma-Aldrich). Total RNA (2 µg) was reverse-transcribed to cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The resulting cDNA was diluted 1:10 with nuclease-free water and used for qRT-PCR. Gene expression for amino acid solute carrier transporters (SLC)-1A5, SLC6A19, SLC7A5, SLC7A1, SLC7A7, SLC7A9, SLC6A14, SLC38A2, and mTOR pathway genes was analyzed using TaqMan® Gene Expression Assays.

Reactions were performed in a 10 μL volume using the CronoSTAR™ Real-Time PCR System (Takara Bio) under standard cycling conditions. β-actin served as the endogenous control, and each sample was tested in duplicate. Non-template controls were included in all runs to verify specificity.

Carcass characteristics

Carcass evaluation was performed following national grading guidelines set by MAFRA (Republic of Korea). Grading was executed by certified livestock evaluators and categorized as 1+, 1, 2, or ungraded. Only graded carcasses were included in the statistical analysis. Carcass weights were taken from the left half of split carcasses, and backfat thickness was calculated as the average of two measurements: between the 11th and 12th thoracic vertebrae, and between the last thoracic and first lumbar vertebrae. A calibrated ruler was used by trained personnel for these measurements.

Statistical analysis

Data were analyzed using the GLM procedure in SAS (SAS Institute, Cary, NC) with a randomized complete block design. Linear and quadratic polynomial contrasts were applied to evaluate the effect of increasing dietary NCG levels (0, 0.4, 0.7, and 1.0 g/kg). Additionally, orthogonal contrasts were used to compare the NP and LP groups. The experimental unit for all statistical analyses was the pen. Statistical significance was considered at $p < 0.05$ or $p < 0.01$, while trends were noted when p-values ranged from 0.05 to 0.10.

RESULTS

Growth performance

In phase 1, BW tended to increase with NCG supplementation (Table 3). In phases 2 and 3, BW showed a linear increase as the dietary NCG level increased. Additionally, the NP group demonstrated greater BW in phases 2 and 3 compared to the LP0 treatment. During phase 1, no effects were observed on ADG or ADFI;

however, G:F was linearly improved with increasing NCG supplementation. Notably, pigs supplemented with 1.0 g/kg NCG achieved a G:F comparable to the NP group in phase 1, suggesting that NCG can partially compensate for the reduced protein level in terms of feed conversion efficiency. The NP group showed a higher G:F and a tendency toward increased ADG compared to LP0. In phase 2, ADG and ADFI remained unaffected, but NCG supplementation continued to linearly improve G:F. The NP group also tended to exhibit a higher G:F compared to LP0. In phase 3, no significant changes were observed in ADG or ADFI, but G:F tended to increase linearly with increasing NCG supplementation. The NP group demonstrated a significantly improved G:F compared to LP0. Overall, the results indicated a linear increase in both overall ADG and G:F with increasing levels of dietary NCG compared to the LP0 treatment. Furthermore, the NP treatment exhibited significantly greater overall ADG and G:F compared to LP0.

Nutrient digestibility and blood urea nitrogen

During phase 1, DM, GE, and EE digestibility remained unaffected by dietary treatments (Table 4). However, CP digestibility tended to increase with increasing NCG supplementation. No significant differences in DM, GE, EE, and CP digestibility were observed between the NP and LP0 treatments. Similar trends were observed in phases 2 and 3, where DM, GE, and EE digestibility remained unchanged, while CP digestibility was linearly improved with NCG supplementation. The serum content of Arg, Leu, Lys, Phe, Trp, Val, Ala, Glu, Gly, Cit, and Orn amino acids was linearly improved with increasing NCG supplementation (Table 5). The serum concentration of His and Thr tended to increase with increasing NCG supplementation. Furthermore, the content of Arg, His, Leu, Val, Glu, and Cit was higher in the NP treatment compared to LP0. Additionally, the serum concentration of Ile, Lys, Met, Phe, Thr, Trp, Gly, and Pro showed a tendency to improve in the NP group relative to LP0. The blood BUN levels decreased linearly with increasing NCG supplementation. However, BUN levels were significantly higher in the NP group compared to LP0.

Amino acid transporters and mTOR expression

Dietary NCG supplementation enhanced the expression of several amino acid transporters in the jejunum (Figure 1). Significant linear increases were observed for SLC6A19, SLC7A5, SLC7A7, SLC7A9, SLC38A2, and mTOR. Expression of SLC1A5 and SLC7A1 also tended to increase, whereas SLC6A14 remained unchanged. The SLC7A9 expression was greater in LP0 pigs than in NP pigs, suggesting a possible adaptive response to reduced amino acid availability under protein restriction.

Carcass traits

The marketing age of pigs was reduced linearly with increasing dietary NCG supplementation (Figure 2). The backfat thickness was linearly reduced, while slaughter weight, and carcass weight were linearly increased with NCG supplementation. Dressing percentage and carcass length remained unaffected. Comparisons between NP and LP0 treatments revealed that NP-fed pigs had a lower marketing age, and a greater carcass weight compared with LP0-fed pigs. However, backfat thickness, carcass length, slaughter weight, dressing percentage, and carcass length showed no differences between the NP and LP0 groups. Although carcass grade distribution was not subjected to statistical analysis, a numerical increase in the proportion of grade 1+ carcasses and a reduction in grade 2 carcasses were observed with increasing NCG supplementation (Figure 3), indicating a potential improvement in carcass quality.

DISCUSSION

The present study demonstrated that dietary supplementation of NCG in low-protein diets resulted in a linear improvement in BW, particularly evident during the later growth phases, and a linear increase in G:F with increasing levels of NCG. These findings indicate that NCG enhances feed efficiency under protein-

restricted conditions, with its effects becoming more pronounced over time. The observed improvements in growth performance are likely mediated through the metabolic role of NCG in stimulating endogenous arginine synthesis via activation of carbamoyl phosphate synthetase I in the urea cycle [11]. Arginine is a key amino acid involved in muscle growth and nutrient utilization, protein synthesis, growth hormone production, insulin secretion, and nitric oxide production [6,15,16]. In LP diets, where dietary arginine and other essential amino acids may be limiting, NCG compensated systemic arginine availability, thereby promoting nitrogen retention and lean tissue accretion. Similar findings have been reported in previous studies that NCG supplementation improved G:F and nitrogen efficiency in growing pigs fed protein-reduced diets [20–22]. In agreement, the increased growth performance and protein accretion in pigs receiving NCG was reported [4,8,17]. The lack of effect on ADFI in our study aligns with these previous results [1,4,7,21] and suggests that NCG does not act as an appetite stimulant but rather improves metabolic efficiency. The greater growth performance observed in the NP group confirms the well-established relationship between adequate dietary protein and growth. However, the fact that NCG supplementation in LP diets partially restored growth and feed efficiency toward levels seen in the NP group emphasizes its practical value as a functional additive for reducing dietary protein levels without compromising performance.

The linear improvement in CP digestibility with increasing NCG supplementation, observed consistently across all growth phases, highlights NCG capacity to enhance nitrogen utilization efficiency in pigs fed low-protein diets. This effect is likely attributed to the role of NCG in stimulating endogenous Arg synthesis, thereby supporting intestinal protein metabolism and mucosal function [3,10,25]. The unchanged DM, GE, and EE digestibility across treatments suggests that NCG's effects are specific to nitrogen metabolism rather than general nutrient absorption. However, it was shown that NCG supplementation increased the metabolizable energy utilization without any effects on CP and EE digestibility in growing pigs fed low protein diets [20,29]. The improvement in CP digestibility, despite no difference between NP and LP0 groups, suggests that NCG supplementation restores protein utilization efficiency to a level comparable

with standard CP diets. This is supported by the enhanced serum levels of both essential and functional amino acids, particularly Arg, His, Leu, Val, and Cit, in response to increasing NCG levels. These increases reflect improved intestinal absorption and reduced catabolism. This is in agreement with previous findings where NCG enhanced amino acid availability in pigs under protein-restricted conditions [1,16,18,21] or in underfed ewe [13]. Moreover, the higher serum levels of Arg, His, Ile, Val, and Glu in the NP group compared to LP0 affirm the deficiency-induced metabolic impact of protein restriction, which NCG appears to counteract partially or fully depending on dosage. The linear reduction in BUN with NCG supplementation further shows improved nitrogen efficiency. Lower BUN levels indicate reduced nitrogen wastage and enhanced retention, which is in agreement with earlier studies where NCG decreased urea formation and nitrogen excretion in pigs [17,25] and ruminants [19]. Interestingly, the significantly higher BUN levels in the NP group compared to LP0 are likely a reflection of increased total nitrogen intake in NP diets.

The linear increase in the expression of SLC6A19, SLC7A5, SLC7A7, SLC7A9, SLC38A2, and mTOR with NCG supplementation reflects a direct effect of NCG on amino acid sensing and absorption in the jejunum. These transporters control the uptake of neutral, cationic, and branched-chain amino acids [17,25]. Their upregulation indicates an improved capacity for intestinal amino acid transport under LP diets when NCG is included. NCG is not rapidly catabolized in the intestine like Arg and can reach enterocytes at higher concentrations [14,25]. As a result, NCG may increase intracellular Arg levels more efficiently than dietary Arg. The higher availability of NCG directly stimulates amino acid-sensing pathways, including mTOR activation [9,10], which requires intracellular amino acids such as Leu and Arg to initiate downstream signaling for protein synthesis and cell proliferation [30,31]. Moreover, NCG enhances antioxidant status in the enterocytes by maintaining higher nitric oxide levels and reducing oxidative stress [10,14,26]. Healthier enterocytes with improved redox balance are likely to express higher levels of nutrient transporters to sustain anabolic and absorptive processes [32]. This improvement in enterocyte function may partly explain the linear upregulation of SLCs observed in this study. The present findings are

consistent with previous studies where NCG supplementation increased the expression of amino acid transporters in the intestine [33]. This molecular change supports the observed improvement in serum amino acid concentrations and CP digestibility. Arg is a central regulator of nutrient signaling [25]. By enhancing endogenous Arg synthesis, NCG likely activates nutrient-sensing pathways that stimulate transporter expression. The increase in mTOR expression also confirms enhanced intracellular amino acid availability. NCG supplementation to underfed ewes helped increase the expression of amino acid transporters, including SLC7A1, SLC1A1, SLC1A5, and SLC15A1 in the duodenum, jejunum, and ileum, and increase the expression of mTOR in the ileum [13]. When mTOR expression increases, protein synthesis and cellular anabolic activity also increase [2,10,19]. This can contribute to better nutrient utilization and growth. Earlier studies also reported that NCG improved mTOR signaling in tissues such as the intestine and muscle in pigs and chickens [10,16]. SLC7A5 is a known transporter for large neutral amino acids such as Leu and Phe [17,24]. Its expression increases under high intracellular Arg or Leu [30,31]. SLC7A7 also facilitates cationic amino acid transport [13,23]. Its response to NCG reflects an increased need for efficient Arg uptake under improved Arg availability. The higher expression of SLC7A9 in the LP0 group compared to NP indicates a possible compensatory mechanism under Arg deficiency. Under low luminal Arg, the jejunum may upregulate specific transporters to improve amino acid salvage. This effect appears to be normalized with NCG supplementation, where Arg sufficiency reduces the need for compensatory overexpression.

The observed linear improvement in carcass weight and loin eye area with increasing NCG supplementation indicates a positive effect on lean tissue deposition under LP conditions. This is likely linked to enhanced amino acid availability and utilization. NCG increases endogenous Arg synthesis, which is known to stimulate protein accretion through mTOR activation and nitric oxide production [5,19,26]. Improved mTOR signaling promotes muscle protein synthesis [2,14,26], which explains the increase in loin eye area and carcass weight in NCG-supplemented pigs. No significant differences were detected in backfat thickness among dietary treatments. This implies that the improved carcass traits were due to lean mass

gain rather than fat deposition. Similar outcomes were reported that NCG enhanced lean tissue accretion without affecting fat content in growing pigs under CP restriction [7,16]. The selective improvement in muscle mass without a concurrent increase in fat may reflect a protein-sparing effect of NCG. Under LP conditions, NCG allows more efficient use of dietary and endogenous amino acids for tissue accretion. The results are consistent with studies in both pigs and poultry, where NCG improved muscle yield and carcass traits under reduced protein intake [1,21,22] or normal diet [7]. These effects were often mediated through enhanced amino acid absorption, increased serum IGF-1, and better nitrogen retention. In the current trial, the improved CP digestibility and reduced BUN further support the efficient use of nitrogen for muscle growth rather than excretion.

CONCLUSION

Dietary supplementation of NCG under low-protein conditions improved CP digestibility, reduced BUN levels, and increased serum amino acid concentrations, reflecting enhanced nitrogen utilization. The upregulation of amino acid transporters and mTOR gene expression in the jejunum indicates that NCG may promote intestinal absorption capacity and support anabolic signaling pathways. These effects may result from greater Arg bioavailability and improved enterocyte function due to better oxidative balance. Furthermore, NCG supplementation partially mitigated the negative effects of dietary protein reduction on carcass characteristics. These findings suggest that supplementation of 1.0 g/kg NCG can be a nutritional strategy to reduce dietary protein levels without compromising growth performance or carcass traits, which is especially relevant for modern fast-growing pigs raised under high-density commercial conditions. Because most measured responses exhibited linear improvements without evidence of quadratic effects, future studies should evaluate higher dietary inclusion levels of NCG to determine the optimal supplementation dose and further elucidate its underlying physiological mechanisms.

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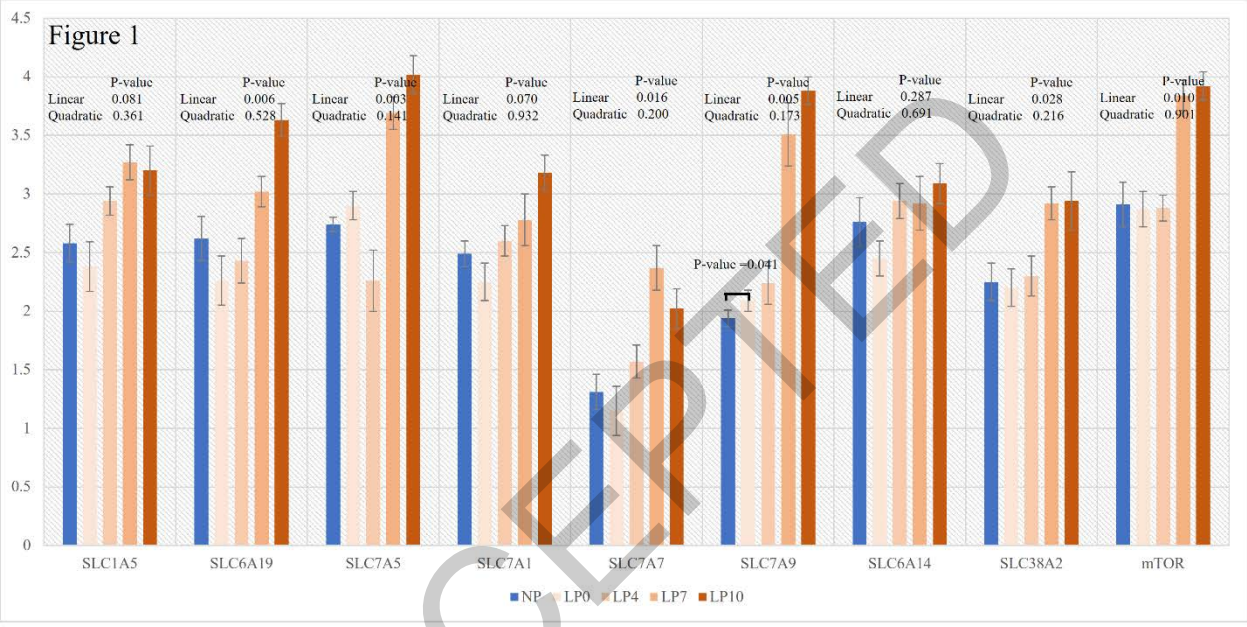
353 **FIGURE LEGENDS**



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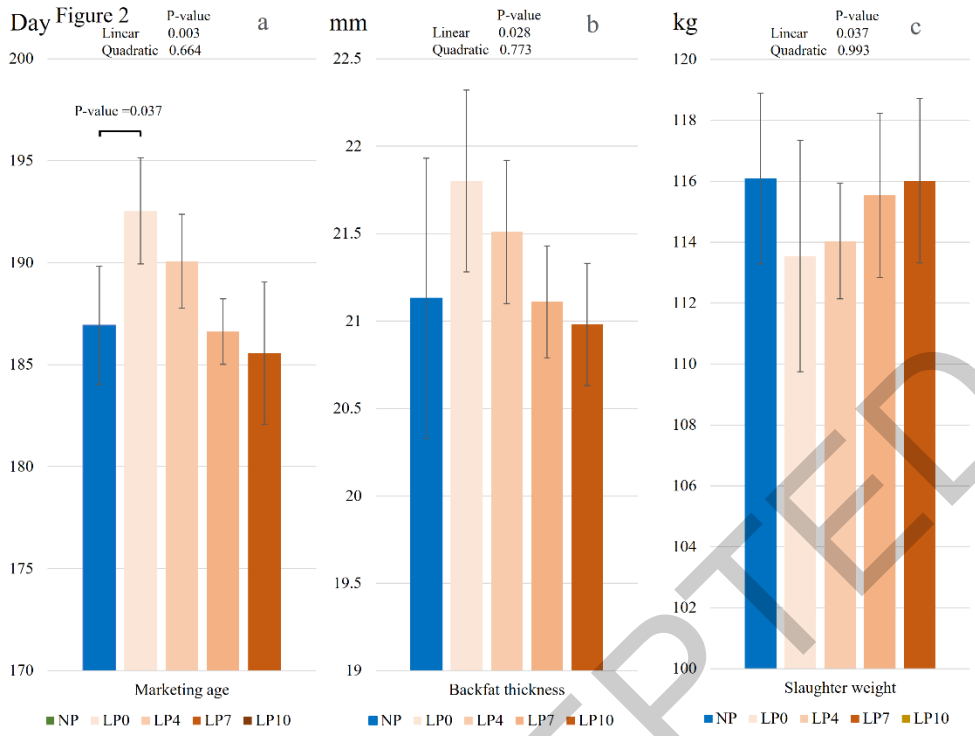
355 Figure 1. Effect of dietary crude protein level and n-carbamylglutamate (NCG) supplementation level on
356 gene experession of solute carrier (SLC) and mechanistic target of rapamycin (mTOR) in the jejunum of
357 growing-finishing pigs. NP, standard protein level; LP0, 2% lower protein level; LP4, 2% lower protein
358 level+ 0.4 g/kg NCG supplementation; LP7, 2% lower protein level+ 0.7 g/kg NCG supplementation; LP10,
359 2% lower protein level+1.0 g/kg NCG supplementation.

360

361 (A) (B) (C)



362

363 (D) (E) (F)

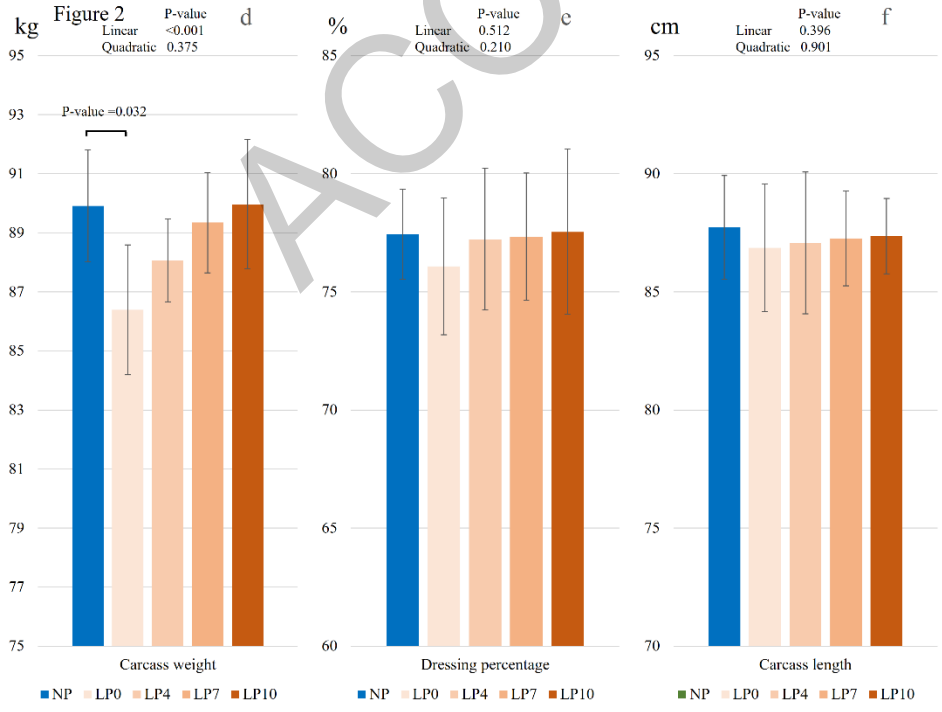


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Figure 2. Effect of dietary crude protein level and n-carbamylglutamate (NCG) supplementation level on carcass traits of growing-finishing pigs. NP, standard protein level; LP0, 2% lower protein level; LP4, 2% lower protein level+ 0.4 g/kg NCG supplementation; LP7, 2% lower protein level+ 0.7 g/kg NCG supplementation; LP10, 2% lower protein level+1.0 g/kg NCG supplementation.

Figure 3

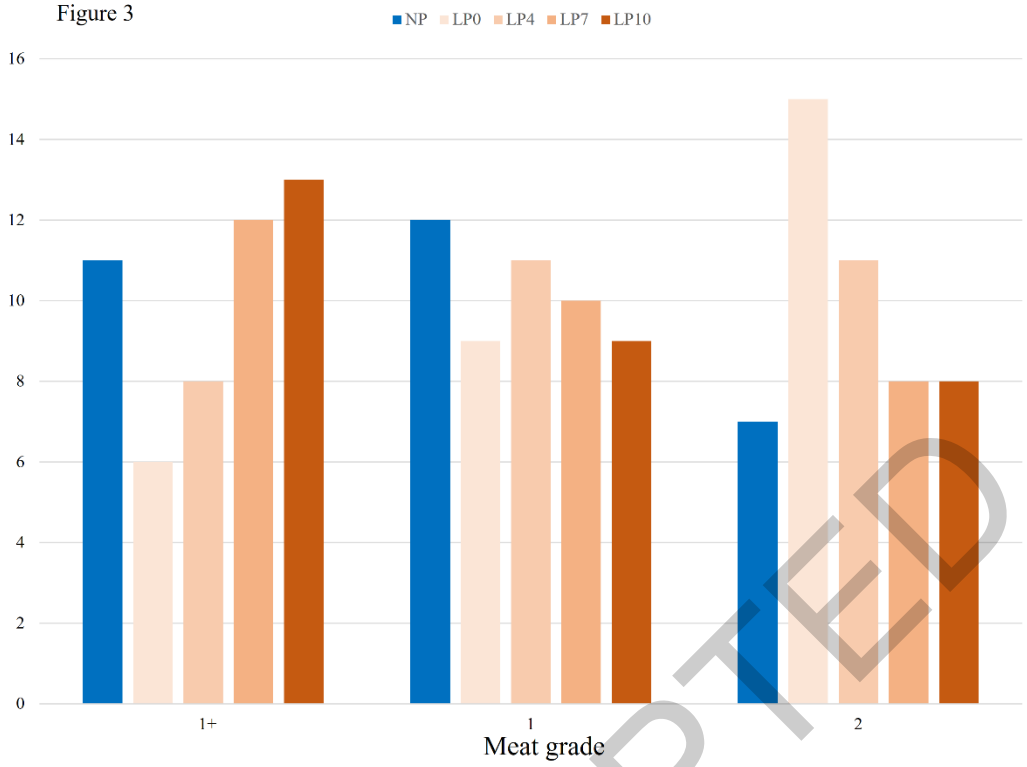

Figure 3. Effect of dietary crude protein level and n-carbamylglutamate (NCG) supplementation level on meat grade of growing-finishing pigs. NP, standard protein level; LP0, 2% lower protein level; LP4, 2% lower protein level+ 0.4 g/kg NCG supplementation; LP7, 2% lower protein level+ 0.7 g/kg NCG supplementation; LP10, 2% lower protein level+1.0 g/kg NCG supplementation.

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

Diet	Standard protein			Low protein		
Protein content (%)	16	15	14	14	13	12
Corn	64.67	67.9	70.92	69.83	73.06	76.07
Soybean meal (44%)	17.59	15.11	12.60	11.88	9.40	6.92
DDGS	10.00	10.00	10.00	10.00	10.00	10.00
Animal fat	2.17	1.84	1.58	2.03	1.71	1.46
Molasses (sugar beet)	3.00	3.00	3.00	3.00	3.00	3.00
$_{L}$-Lys (78.8%)	0.32	0.27	0.22	0.50	0.44	0.39
$_{DL}$-Met (98%)	0.03	-	-	0.08	0.05	0.02
Thr (99%)	0.04	0.02	0.01	0.12	0.10	0.09
Trp (10%)	0.14	0.06	0.08	0.43	0.34	0.36
Limestone	0.78	0.68	0.61	0.79	0.70	0.63
MDCP	0.56	0.42	0.28	0.64	0.50	0.36
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride (50%)	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[1]	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix[2]	0.15	0.15	0.15	0.15	0.15	0.15
Phytase	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100
Calculated composition (%)						
ME (kcal/kg)	3,300	3,300	3,300	3,300	3,300	3,300
Crude protein	16.00	15.00	14.00	14.00	13.00	12.00
Crude fat	4.74	4.47	4.26	4.64	4.41	4.21
Calcium	0.59	0.52	0.46	0.59	0.52	0.46
Digestible phosphorus	0.27	0.24	0.21	0.27	0.24	0.21
Lys	0.96	0.85	0.75	0.94	0.84	0.73
SID Lys	0.85	0.75	0.65	0.85	0.75	0.65
SID Met	0.26	0.22	0.21	0.29	0.25	0.21
SID Met+Cys	0.48	0.43	0.41	0.48	0.43	0.38
SID Thr	0.52	0.47	0.43	0.52	0.47	0.43
SID Trp	0.15	0.13	0.12	0.15	0.13	0.12
SID Val	0.65	0.61	0.57	0.55	0.51	0.47
SID Ile	0.56	0.52	0.48	0.46	0.42	0.38
SID Leu	1.28	1.23	1.18	1.15	1.10	1.04
SID His	0.38	0.36	0.34	0.33	0.31	0.28
SID Phe	0.68	0.64	0.59	0.58	0.53	0.49
SID Phe +Tyr	1.18	1.00	1.03	1.00	0.93	0.85
SID Arg	0.86	0.79	0.72	0.69	0.62	0.55

[1]Supplied per kilogram of mineral premix: 80,000 mg Fe, 170 mg Co, 8,500 mg Cu, 25,000 mg Mn, 95,000 mg Zn, 140 mg I, 150 mg Se.

[2]Supplied per kilogram of vitamin premix: 12,000,000 IU vitamin A, 2,400,000 IU vitamin D_3, 132,000 IU vitamin E, 1,500 mg vitamin K3, 3,000 mg vitamin B_1, 11,250 mg vitamin B_2, 3,000 mg vitamin B_6, 45 mg vitamin B_{12},

36,000 mg pantothenic acid, 30,000 mg niacin, 600 mg biotin, 4,000 mg folic acid.

DDGS, dried distiller's grains with solubles; MDCP, mono-di calcium phosphate; ME, metabolizable energy; SID, standardized ileal digestibility.

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Table 2. Primer sequences used for real-time PCR.

Target Gene	Accession No.	Primer Sequence (5'-3')	Product Size (bp)
β-actin	DQ452569.1	Forward: TGCGGGACATCAAGGAGAA Reverse: GCCATCTCCTGCTCGAAGTC	58
SLC1A5	XM_003127238.4	Forward: CCCTTCCCACACTCGACTGA Reverse: CCCGGACCTAGCCTCTTGA	57
SLC6A19	XM_003359855.3	Forward: GCAACGTGACGCAGGAGAA Reverse: GGTCGGAGGCGTTGCA	57
SLC7A5	NM_003486	Forward: CTCTTCCTGATCGCCGTCTC Reverse: CTTCTGACACAGGACGGTCGT	165
SLC7A1	NM_001012613.1	Forward: CATCTTTGCCGTGATCATAATTCT Reverse: TTTGTTGACCATGGCTGACTCT	79
SLC7A7	NM_001110421.1	Forward: TTTGGTTCCCAAGGTTGCA Reverse: GCAGCTTCCTGGCATTGC	58
SLC7A9	EF127857.1	Forward: TTGCCATCATCTGTCTCAGCTT Reverse: GCTGCAGCCTGCGTAGAAG	62
SLC6A14	GQ387269	Forward: TCCAGAAGCTCTAGCCCAACT Reverse: CAAAACCAAGCAGCAACCC	190
SLC38A2	NM_018976	Forward: GTTACCTTTGGTGATCCAGGC Reverse: ACCAATGACACCAGCAGAACC	96
mTOR	XM_003127584.4	Forward: TTGTTGCCCCCTATTGTGAAG Reverse: CCTTTCGAGATGGCAATGGA	61

SLC, solute carrier transporters; mTOR, mechanistic target of rapamycin.

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Table 3. Effect of dietary crude protein level and n-carbamylglutamate (NCG) supplementation level on growth performance in growing-finishing pigs.

Protein level	NP	LP				SEM	P-value		
NCG, g/kg	0	0	0.4	0.7	1.0		NP vs LP0	NCG levels	
								Linear	Quadratic
BW, kg									
Initial	50.83	50.78	51.00	50.91	50.72	0.38	0.896	0.826	0.447
Phase 1	69.42	68.12	68.44	68.82	69.24	0.71	0.107	0.090	0.915
Phase 2	88.71	86.53	86.98	87.49	88.03	0.66	0.024	0.010	0.904
Final	108.19	104.81	105.63	106.57	107.45	0.56	<0.001	<0.001	0.943
Phase 1 (d 1-21)									
ADG, kg	0.88	0.83	0.83	0.85	0.88	0.04	0.098	0.124	0.647
ADFI, kg	1.76	1.74	1.75	1.73	1.73	0.05	0.650	0.764	0.909
G:F	0.50	0.47	0.47	0.49	0.51	0.01	0.025	<0.001	0.196
Phase 2 (d 22-42)									
ADG, kg	0.92	0.88	0.88	0.89	0.89	0.03	0.210	0.507	0.995
ADFI, kg	1.85	1.85	1.84	1.82	1.83	0.05	0.977	0.440	0.826
G:F	0.49	0.47	0.48	0.49	0.49	0.01	0.071	0.017	0.617
Phase 3 (d 43-63)									
ADG, kg	0.93	0.87	0.89	0.91	0.92	0.04	0.207	0.106	0.973
ADFI, kg	2.03	2.03	2.05	2.02	2.05	0.05	0.953	0.902	0.944
G:F	0.46	0.43	0.43	0.45	0.45	0.01	0.010	0.085	0.901
Overall (d 1-63)									
ADG, kg	0.91	0.89	0.87	0.88	0.90	0.01	<0.001	0.003	0.696
ADFI, kg	1.88	1.88	1.88	1.86	1.87	0.02	0.776	0.515	0.988
G:F	0.48	0.46	0.46	0.47	0.48	0.01	<0.001	<0.001	0.791

[1]NP, standard protein (16-14%); LP, with 2% lower protein.

SEM, standard error of the mean; BW, body weight; ADG, average daily weight gain; ADFI, average daily feed intake; G:F, feed efficiency.

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Table 4. Effect of dietary crude protein level and n-carbamylglutamate (NCG) supplementation level on the nutrient digestibility of growing-finishing pigs.

Protein level	NP	LP				SEM	P-value		
NCG, g/kg	0	0	0.4	0.7	1.0		NP vs LP0	NCG levels	
								Linear	Quadratic
Phase 1 (d 21)									
Dry matter	87.22	86.00	86.53	86.86	87.09	1.13	0.209	0.355	0.865
Gross energy	88.42	87.34	87.36	87.89	87.93	1.26	0.306	0.288	0.598
Crude protein	87.02	85.41	85.82	86.60	87.02	0.96	0.128	0.085	0.999
Ether extract	70.18	68.29	68.32	68.51	68.64	1.32	0.225	0.746	0.958
Phase 2 (d 42)									
Dry matter	85.99	84.52	85.25	86.22	86.77	1.60	0.537	0.108	0.978
Gross energy	86.45	85.51	85.81	86.02	86.28	1.42	0.556	0.570	0.984
Crude protein	86.03	84.15	84.81	85.66	86.66	1.09	0.130	0.047	0.864
Ether extract	68.64	66.96	67.01	67.19	67.98	1.26	0.185	0.424	0.686
Phase 3 (d 63)									
Dry matter	83.65	82.70	82.71	83.11	83.34	1.24	0.495	0.547	0.897
Gross energy	83.07	82.07	82.29	82.49	82.85	0.99	0.441	0.362	0.904
Crude protein	82.98	81.30	81.57	82.81	83.12	0.85	0.090	0.050	0.828
Ether extract	67.12	66.36	66.73	66.87	67.00	1.21	0.552	0.552	0.881

[1]NP, standard protein (16-14%); LP, with 2% lower protein.

SEM, standard error of the mean.

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Table 5. Effect of dietary crude protein level and n-carbamylglutamate (NCG) supplementation level on serum amino acid levels and urea nitrogen levels of finishing pig.

Protein level	NP	LP				SEM	P-value		
NCG, g/kg	0	0	0.4	0.7	1.0		NP vs LP0	NCG levels	
								Linear	Quadratic
Indispensable AA, nmol/mL									
Arginine	292.99	257.75	278.06	300.64	309.97	9.88	0.005	<0.001	0.450
Histidine	102.19	91.66	94.44	97.41	100.96	5.01	0.041	0.053	0.910
Isoleucine	170.44	147.52	151.68	154.27	162.70	8.93	0.052	0.917	0.391
Leucine	279.83	256.56	259.36	267.25	276.68	7.81	0.021	0.005	0.502
Lysine	233.59	220.73	231.92	234.23	242.81	9.19	0.089	0.024	0.945
Methionine	163.99	134.07	135.19	156.83	158.67	8.86	0.094	0.261	0.852
Phenylalanine	110.42	93.33	97.07	102.90	109.06	7.45	0.053	0.022	0.803
Threonine	177.51	154.19	164.36	172.68	178.99	7.28	0.097	0.071	0.978
Tryptophan	78.92	68.97	73.15	76.68	78.59	4.87	0.053	0.050	0.747
Valine	334.30	300.73	301.01	315.09	319.42	12.22	0.004	0.004	0.319
Dispensable AA, nmol/mL									
Alanine	455.78	435.22	446.76	453.00	454.91	15.05	0.193	0.162	0.637
Aspartate	40.53	36.38	38.58	39.73	41.11	5.30	0.470	0.358	0.912
Glutamate	221.51	183.91	252.08	270.83	315.78	11.67	0.003	<0.001	0.186
Glutamine	340.15	316.83	328.51	335.14	339.75	11.67	0.077	0.044	0.658
Glycine	902.61	832.70	840.24	874.27	927.71	31.07	0.078	0.003	0.290
Serine	79.37	70.30	71.03	73.92	77.76	5.58	0.084	0.114	0.702
Tyrosine	105.33	97.59	98.39	100.98	104.02	6.11	0.244	0.155	0.944
Proline	417.32	386.11	395.46	399.06	404.33	14.76	0.065	0.220	0.844
Cysteine	51.08	45.19	47.77	49.40	51.89	5.14	0.211	0.195	0.991
Citruline	225.68	202.64	236.36	262.72	308.43	9.99	0.020	<0.001	0.402
Ornithine	176.46	166.89	180.66	191.13	220.42	7.21	0.117	<0.001	0.159
Urea nitrogen, mmol/L	2.321	1.976	1.580	1.194	1.037	0.105	0.027	<0.001	0.054

[1]NP, standard protein (16-14%); LP, with 2% lower protein.

SEM, standard error of the mean; AA, amino acids.

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