Effects of Glycine Supplementation in Low-Protein Diets on Growth Performance, Carcass Composition, and Blood Biochemical Parameters of Broiler Chickens: Postprint

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Abstract

This study aimed to investigate the effects of dietary glycine (Gly) supplementation in low crude protein (CP) diets on growth performance, carcass composition, and blood biochemical indices in broiler chickens. A total of 180 one-day-old healthy Arbor Acres (AA) male broiler chicks were randomly allocated to 3 groups with 6 replicates per group and 10 birds per replicate. The three groups were: positive control (PC) group with dietary CP levels of 22.0% and 20.0% for the starter and finisher phases, respectively; negative control group with dietary CP levels of 18.0% and 15.5% for the starter and finisher phases, respectively; and Gly group, which received the negative control diet supplemented with Gly to achieve dietary Gly and serine levels of 2.32%. The experimental period lasted 42 days, divided into two phases: starter (1–21 d) and finisher (22–42 d). The results showed that: 1) Reducing dietary CP level by 4.0–4.5 percentage points significantly increased feed-to-gain ratio (F/G) during 1–21 d ($P<0.05$) and significantly decreased average daily gain (ADG) during 22–42 d ($P<0.05$); Gly supplementation significantly decreased F/G during 1–21 d ($P<0.05$) and significantly increased ADG during 22–42 d ($P<0.05$), achieving growth performance similar to the PC group. 2) CP reduction decreased breast muscle percentage by 9.5% ($P<0.05$) and increased abdominal fat percentage by 60.3% ($P<0.05$) at 42 days of age; Gly supplementation increased breast muscle percentage by 17.6% ($P<0.05$) and decreased abdominal fat percentage by 34.6% ($P<0.05$), resulting in carcass composition similar to the PC group. Neither CP reduction nor Gly supplementation significantly affected dressing percentage, eviscerated yield percentage, or leg muscle percentage ($P>0.05$). 3) No significant differences in blood biochemical indices were observed among groups ($P>0.05$). In conclusion, Gly supplementation in low-CP diets can im-

prove growth performance and carcass composition, suggesting the applicability of Gly in low-CP diets.

Full Text

Effects of Glycine Addition in Low Crude Protein Diets on Performance, Carcass Composition and Blood Biochemical Parameters of Broilers

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Abstract

This experiment investigated the effects of glycine (Gly) supplementation in low crude protein (CP) diets on growth performance, carcass composition, and blood biochemical parameters of broiler chickens. A total of 180 one-day-old healthy male Arbor Acres broiler chicks were randomly allocated into three groups, each consisting of six replicates with ten birds per replicate. The three dietary treatments were: positive control (PC) group with 22.0% and 20.0% CP in starter and grower phases, respectively; negative control (NC) group with 18.0% and 15.5% CP in starter and grower phases; and Gly group, which was supplemented with Gly on top of the NC diet to achieve 2.32% Gly+serine (Ser). The 42-day experiment was divided into two phases: starter (1–21 days) and grower (22–42 days). The results showed: (1) Reducing dietary CP by 4.0–4.5 percentage points significantly increased feed-to-gain ratio (F/G) during days 1-21 ($P<0.05$) and decreased average daily gain (ADG) during days 22-42 ($P<0.05$). Gly supplementation significantly reduced F/G during days 1-21 ($P<0.05$) and increased ADG during days 22-42 ($P<0.05$), restoring growth performance to levels comparable to the PC group. (2) Low CP diet decreased breast muscle percentage by 9.5% ($P<0.05$) and increased abdominal fat percentage by 60.3% ($P<0.05$) at 42 days of age. Gly supplementation significantly increased breast muscle percentage by 17.6% ($P<0.05$) and reduced abdominal fat percentage by 34.6% ($P<0.05$), yielding carcass composition similar to the PC group. Neither CP reduction nor Gly supplementation significantly affected slaughter percentage, dressing percentage, or leg muscle percentage ($P>0.05$). (3) No significant differences in blood biochemical parameters were observed among groups ($P>0.05$). In conclusion, Gly supplementation in low CP diets can improve growth performance and carcass composition, demonstrating its

promising applicability in low CP feeding strategies for broilers.

Keywords: broiler; low crude protein diet; glycine; growth performance; carcass composition

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In recent years, China has faced a shortage of protein feed resources, requiring substantial annual imports of soybean meal and fish meal. Meanwhile, the low utilization efficiency of dietary crude protein (CP) by livestock and poultry results in substantial nitrogen excretion in manure, exacerbating environmental burdens. Currently, nutritional modulation strategies involving supplementation of synthetic amino acids (AA) in low CP diets have garnered significant attention in animal nutrition research, as they can maintain animal performance while improving CP utilization and reducing dietary protein ingredient usage.

To eliminate differences in AA digestibility among feed ingredients, low CP diet formulation is transitioning from the "ideal protein pattern" to the "ideal digestible AA pattern" based on digestible AA. The ideal protein AA pattern is a requirement model where various AAs maintain constant ratios relative to lysine (Lys). Glycine (Gly), also known as 2-aminoacetic acid, is an essential AA for poultry. Beyond serving as a precursor for protein synthesis, Gly functions as a functional AA involved in synthesizing creatine [1], uric acid (UA) [2], glutathione [3], and heme [4], while also exerting immunomodulatory, cytoprotective [5], and antioxidant effects [6]. Previous studies indicate that reducing dietary CP beyond certain limits impairs performance in livestock and poultry [7-8], likely due to deficiency of specific nutrients. In low CP diets, Gly is a limiting AA [9-10]. Existing research on Gly supplementation in low CP diets has primarily focused on the early growth phase of broilers, with limited studies on later phases or the entire production period. This experiment examined the effects of Gly supplementation in low CP diets on growth performance, carcass composition, and blood biochemical parameters of broilers from 1 to 42 days of age.

1.1 Experimental Material

Crystalline Gly was purchased from Hebei Hemei Amino Acid Co., Ltd. with a purity of 99%.

1.2 Experimental Design and Diet Composition

The experiment utilized 180 one-day-old healthy male Arbor Acres broiler chicks with similar body weight, randomly divided into three groups with six replicates per group and ten birds per replicate. The 42-day trial consisted of starter (1–21 days) and grower (22–42 days) phases. The PC group received diets with 22.0% and 20.0% CP in starter and grower phases, respectively, while the negative control (NC) group received 18.0% and 15.5% CP. The Gly group was supplemented with Gly on top of the NC diet to achieve 2.32% Gly+Ser.

Basal diets were formulated according to NRC (1994), "Feeding Standard of Chickens" (NY/T 33–2004), and an ideal digestible AA pattern [recommended by Adisseo, Table 1]. The composition and nutrient levels of basal diets are presented in Table 2 . This AA pattern was identical for both growth phases, as established by Adisseo for simplified practical application. Basal diets were cold-pelleted and fed as pellets.

Table 1 The Ideal Model of Digestible Amino Acids

Item	Ratio
Lys	100
Met+Cys (PC group)	72
Met+Cys (NC group)	76
Thr	65
Trp	16
Val	75
His	35
Phe+Tyr	115
Arg	105
Ile	68
Leu	110

Table 2 Composition and Nutrient Levels of Basal Diets (Air-Dry Basis), %

Item	1-21 Days	22-42 Days
	PC	NC
Ingredients		
Corn	52.90	62.40
Soybean meal	38.00	27.50
Vegetable oil	4.00	4.50
$CaHPO_4$	1.80	1.80
Limestone	1.30	1.30
NaCl	0.30	0.30
DL-Met (99%)	0.22	0.28
L-Lys • HCl (99%)	0.30	0.45
L-Thr (98%)	0.20	0.28
L-Arg • HCl (99%)	0.20	0.35
L-Val (99%)	0.15	0.25
L-Ile (98%)	0.15	0.25
L-His (99%)	0.08	0.15
L-Trp (99%)	0.04	0.06
L-Phe (99%)	0.10	0.15
Leu (99%)	0.20	0.30
Gly (99%)	–	–

Item	1-21 Days	22-42 Days
Vitamin premix[1]	0.03	0.03
Mineral premix[2]	0.20	0.20
50% choline chloride	0.20	0.20
Celite	0.95	0.90
Total	100.00	100.00
Nutrient levels[3]		
ME (MJ/kg)	12.77	12.77
CP	22.24 (22.56)	18.02 (17.89)
Ca	1.10 (1.23)	1.10 (1.24)
AP	0.51 (0.54)	0.61 (0.63)
Lys	1.33 (1.37)	1.22 (1.26)
Met	0.51 (0.54)	0.61 (0.63)
Met+Cys[4]	0.79 (0.83)	0.84 (0.86)
Trp	0.28 (0.29)	0.23 (0.23)
Thr	0.92 (1.03)	0.89 (0.95)
Val	1.10 (1.23)	1.10 (1.24)
Arg	1.33 (1.37)	1.22 (1.26)
Ile	0.87 (0.89)	0.77 (0.79)
Leu	1.64 (1.75)	1.28 (1.47)
His	0.52 (0.58)	0.44 (0.51)
Phe+Tyr	1.67 (1.82)	1.24 (1.39)
Gly+Ser[5]	1.82 (1.92)	1.33 (1.45)

[1]Vitamin premix provided per kg of diet: VA 12,500 IU, VD_3 2,500 IU, VE 50 mg, VK_3 2.65 mg, VB_1 2.0 mg, VB_2 6.0 mg, VB_{12} 0.025 mg, VB_6 3.0 mg, pantothenic acid 12 mg, niacin 50 mg, biotin 0.0325 mg, folic acid 1.25 mg.

[2]Mineral premix provided per kg of diet: Fe 80 mg, Cu 8 mg, Zn 75 mg, Mn 100 mg, I 0.35 mg, Se 0.15 mg.

[3]Nutrient levels are calculated digestible AA values; values in parentheses are measured total AA values.

[4]Based on previous studies in this laboratory: 0.72 for PC group, 0.76 for NC group.

[5]According to Dean et al. [9].

1.3 Management Practices

Birds were raised in four-tier cages with 23 hours of lighting at 30 lx intensity. House temperature was maintained at 30°C during the first week and gradually decreased to 25°C by week 4. Birds had ad libitum access to feed and water throughout the experiment. Management procedures followed the Arbor Acres Broiler Management Handbook.

1.4.1 Growth Performance

At days 1, 21, and 42 (before 09:00), birds were weighed by replicate after overnight fasting. Feed intake was recorded for each replicate to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.4.2 Carcass Composition

On day 42, two birds per replicate with body weight close to the replicate average were selected for slaughter. Breast muscle, leg muscle, and abdominal fat were separated to calculate slaughter percentage, dressing percentage, breast muscle percentage, leg muscle percentage, and abdominal fat percentage.

1.4.3 Blood Biochemical Parameters

On days 21 and 42, two birds per replicate with body weight close to the replicate average were selected for blood collection from the wing vein using anticoagulant tubes. Plasma was harvested by centrifugation at 3,000 r/min for 10 minutes, aliquoted, and stored at -20°C. Total protein (TP), albumin (ALB), uric acid (UA), and urea nitrogen (UN) concentrations were determined using an automatic biochemical analyzer with kits purchased from Shanghai Kehua Bioengineering Co., Ltd.

1.5 Statistical Analysis

Data were analyzed using one-way ANOVA procedure of SPSS 16.0 software. Covariance analysis was used for growth performance during days 22–42. Duncan' s multiple range test was used to detect significant differences, with $P<0.05$ as the significance threshold. Results are expressed as "mean ± standard deviation."

2.1 Effects of Gly Supplementation in Low CP Diets on Broiler Growth Performance

As shown in Table 3 , during days 1–21, the NC group exhibited significantly higher F/G compared to PC and Gly groups ($P<0.05$), while no significant difference was observed between Gly and PC groups ($P>0.05$). The Gly group showed a tendency for higher body weight at 21 days compared to the NC group ($P=0.098$). During days 22–42, no significant differences were detected in ADFI or F/G among groups ($P>0.05$), but ADG in the NC group was significantly lower than in PC and Gly groups ($P<0.05$), with no difference between Gly and PC groups ($P>0.05$). Over the entire experimental period (days 1–42), ADG in Gly and PC groups was similar and significantly higher than in the NC group ($P<0.05$). For F/G, the NC group was significantly higher than the PC group ($P<0.05$), while the Gly group did not differ significantly from the PC group ($P>0.05$). These results indicate that reducing dietary CP impaired broiler growth performance, while Gly supplementation mitigated these negative effects. Gly showed pronounced effects in improving F/G during the starter phase and ADG during the grower phase.

Table 3 Effects of Gly Addition in Low CP Diets on Growth Performance of Broilers

Item	PC	NC	Gly
1-21 days			
Final weight (g)	905.09\$±29.96|877.67±44.76|922.57±22.13||$ADG(g)$|40.49±1.20|39.69±2.38|41.39±0. $*22–42days*$ $*\|\|Finalweight(g)\|2,806.10±141.50\|2,682.16±155.07\|2,732.88±151.65\|\|ADG(g)\|88.8$ $*1–42days*$ $*\|\|ADG(g)\|62.69±3.10^a\|56.63±4.20^b\|61.08±1.65^a\|\|ADFI(g)\|105.42±3.59\|103.44±6.4$		

, Values within the same row with different superscripts differ significantly (P<0.05). The same applies below.

2.2 Effects of Gly Supplementation in Low CP Diets on Broiler Carcass Composition

As presented in Table 4 , neither dietary CP reduction nor Gly supplementation significantly affected leg muscle percentage, slaughter percentage, or dressing percentage at 42 days of age (P>0.05). The NC group exhibited significantly lower breast muscle percentage compared to the PC group (P<0.05), while Gly supplementation significantly increased breast muscle percentage relative to the NC group (P<0.05) to a level comparable with the PC group (P>0.05). The NC group showed significantly higher abdominal fat percentage than the PC group (P<0.05), whereas Gly supplementation significantly reduced abdominal fat percentage compared to the NC group (P<0.05), restoring it to PC group levels (P>0.05). These findings demonstrate that low CP diets decreased breast muscle percentage and increased abdominal fat deposition without affecting leg muscle percentage, slaughter percentage, or dressing percentage. Gly supplementation increased breast muscle percentage and decreased abdominal fat percentage, similarly without affecting the other carcass traits.

Table 4 Effects of Gly Addition in Low CP Diets on Carcass Composition of Broilers at 42 Days of Age, %

Item	PC	NC	Gly
Slaughter percentage	89.30\$±1.48|89.24±1.94|90.06±1.46||$Dressingpercentage$|74.03±1.72|73.30±1.73|73.		

2.3 Effects of Gly Supplementation in Low CP Diets on Blood Biochemical Parameters

As shown in Table 5 , no significant differences in blood biochemical parameters were observed among groups at 21 days of age (P>0.05), though UA concentration in the NC group was numerically lower than in PC and Gly groups. At 42 days of age, TP and ALB concentrations did not differ significantly among groups (P>0.05), but PC group values were numerically higher than NC and Gly groups, with Gly group values higher than NC group. For UA concentration, the NC group was lower than the PC group (P=0.059), and Gly supplementation tended to increase UA concentration (P=0.059) though it remained below PC group levels. UN concentration did not differ significantly among groups (P>0.05). These results suggest that low CP diets tended to decrease UA concentration without affecting UN concentration, while Gly supplementation tended to increase UA concentration. At 42 days of age, TP and ALB concentrations were lower in low CP diets compared to normal CP diets but were improved by Gly supplementation.

Table 5 Effects of Gly Addition in Low CP Diets on Blood Biochemical Parameters of Broilers

Item	21 Days of Age			42 Days of Age		
	PC	NC	Gly	PC	NC	Gly
TP (g/L)	31.77±4.56	33.03±2.29	30.68±1.04	36.38±7.04	33.57±5.23	35.99±6.01
$ALB (g/L)$	16.36±1.56	[illegible]				

Discussion

In 1994, Baker et al. [11] proposed an ideal AA pattern for broilers based on true digestible AA, establishing a foundation for research on broiler AA requirements. In normal CP diets, only Lys, methionine (Met), and threonine (Thr) need supplementation, as other essential AAs exceed ideal protein pattern requirements, resulting in wasteful excretion of excess AAs. The ratio used in this experiment, recommended by Adisseo, closely approximates Baker' s pattern and has been validated repeatedly in our laboratory.

Gly is generally considered an essential or conditionally essential AA for early growth in poultry. Supplementation of synthetic AAs in low CP diets can promote broiler growth [12], but performance is impaired when CP is reduced by more than 3 percentage points [13], likely due to underestimation of specific AAs that become limiting. Previous research investigating whether supplementation of different non-essential AAs in low CP diets could eliminate growth inhibition found that Gly promoted growth to achieve performance equivalent to normal CP diets, whereas glutamic acid (Glu), alanine (Ala), aspartic acid (Asp), and proline (Pro) did not [9,14]. The type and sequence of limiting AAs requiring supplementation in low CP diets depend on dietary composition [15] and CP level [10]. Studies on low CP diets for broilers indicate that after Met (TSAA), Lys, Thr, valine (Val), and isoleucine (Ile) become limiting, further CP reduction impairs performance even with arginine (Arg), tryptophan (Trp), and histidine (His) supplementation, but performance can be improved by Gly addition [10]. This experiment similarly demonstrated that Gly supplementation significantly improved broiler growth in low CP diets, confirming Gly as a limiting AA in such formulations. Previous Gly research in broilers has primarily focused on the starter phase, with limited studies on the grower phase. Our findings indicate that Gly improved feed efficiency during the starter phase and ADG during the grower phase, suggesting its importance throughout the entire production period. The growth-promoting effects of Gly in low CP diets may relate to its role as a limiting AA in protein synthesis and as a substrate in Met metabolism for cysteine (Cys) synthesis, promoting AA balance [16].

Gly requirements in broiler diets are typically expressed as total Gly+Ser. NRC (1994) recommends 1.25% Gly+Ser for normal CP diets, which is clearly inadequate for low CP formulations. Corzo et al. [4] demonstrated that the 1.25% recommendation cannot maintain maximal performance in low CP diets. Waguespack et al. [17] found through three feeding trials that 2.10% Gly+Ser was required for optimal feed efficiency in low CP diets. Dean et al. [9] reported that Gly+Ser levels of at least 2.32% in low CP diets yielded optimal performance. In this experiment, direct Gly supplementation to achieve 2.32% Gly+Ser in low CP diets significantly improved feed efficiency during the starter phase and body weight gain during the grower phase.

3.2 Effects of Gly Supplementation in Low CP Diets on Broiler Carcass Composition

Research has shown that dietary CP level significantly affects carcass composition in meat poultry. Reducing dietary CP decreases breast meat yield [18], breast muscle percentage [19], and CP content in breast muscle while increasing crude fat content [20], and increases abdominal fat deposition [19]. The increased abdominal fat in low CP diets supplemented with crystalline AAs may relate to reduced energy expenditure. AA-balanced diets decrease excess nitrogen excretion as UA, reducing energy consumption and allowing surplus energy to be converted to abdominal fat [13,18].

In this experiment, low CP diets decreased breast muscle percentage and increased abdominal fat percentage at market age, while Gly supplementation increased breast muscle percentage and decreased abdominal fat percentage. The reduction in abdominal fat by Gly supplementation may also relate to its effect on leptin secretion. Studies in mice demonstrated that Gly increased leptin levels, thereby reducing fat synthesis and promoting fat degradation [21]. Additionally, Gly is a crucial component of bile acids in the intestinal lumen, promoting long-chain fatty acid absorption [22], which are less likely to form body fat deposits. In vitro studies with intestinal epithelial cells showed that

Gly promoted cell growth, accelerated protein synthesis, and reduced protein degradation [6], thereby decreasing fat content. The finding that Gly reduces abdominal fat deposition in low CP diets provides important insights for mitigating the adverse effects of low CP diets on broiler carcass composition.

3.3 Effects of Gly Supplementation in Low CP Diets on Blood Biochemical Parameters

Plasma TP and ALB are indicative markers of protein metabolism status, influenced by protein synthesis and degradation rates as well as intake and excretion. UA and UN are end products of protein metabolism in avian species, and their concentrations reflect protein metabolism and nutritional status: low concentrations suggest dietary protein deficiency, while high concentrations indicate low protein utilization efficiency. This experiment showed that Gly supplementation in low CP diets did not significantly affect plasma TP, ALB, or UN concentrations but tended to increase plasma UA concentration during the grower phase, likely because Gly is an important substrate for UA synthesis [4].

Conclusions

1. Gly supplementation in low CP diets improved feed efficiency during the starter phase and increased daily gain during the grower phase, achieving overall growth performance comparable to normal CP diets.
2. Gly supplementation in low CP diets increased breast muscle percentage and decreased abdominal fat percentage at 42 days of age without significantly affecting dressing percentage or leg muscle percentage.
3. Gly supplementation in low CP diets did not significantly affect blood biochemical parameters in broilers.

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