Response of broilers to gradual dietary protein reduction with or without an adequate glycine plus serine level

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ABSTRACT

This study was aimed to probe broiler responses to the maintenance of adequate level of glycine plus serine in low crude protein (CP) diets by glycine (Gly) addition from 8 to 42 days of age. A total of 1152 7-d-old birds were divided into 8 groups and fed with one of the following diets: (I) normal CP diet; (II, III vIV V) diets with 1.5%, 3%, 4.5% and 6% points less CP than normal diet, respectively; (VI, VII, VIII) diets with 3%, 4.5% and 6% points reduced CP than the normal CP diet, respectively, and added with Gly to achieve 2.32% total Gly\textsuperscript{+}Ser. Each treatment was replicated with 12 cages. Results showed that dietary CP reduction by 3% or higher compromised \((p < .05)\) growth performance and carcase traits of broilers. Gly fortification in the diet with a 4.5% CP reduction produced similar accumulative growth performance relative to normal-CP group. When Gly fortification in the diet with a 6% CP reduction, the accumulative growth performance were improved \((p < .05)\) but were still inferior \((p < .05)\) to those in normal-CP group. Gly fortification supported a similar \((p > .05)\) abdominal fat percentage of birds fed the diet containing 4.5% points less CP as compared with birds fed the normal-CP diet. Comparatively, Gly fortification in the diet with a 6% CP reduction resulted in breast muscle yield, serum UN content on d 21 and albumin content on d 42 comparable to \((p > .05)\) those in normal-CP group. Collectively, the absence of Gly addition allowed dietary CP reduction by no more than 3% to obtain normal performance and carcase traits of broilers. Gly fortification in the diet with a 3% CP reduction induced no improvements in growth performance and carcase traits of broilers. However, maintain a level of 2.32% total Gly\textsuperscript{+}Ser via Gly fortification enabled a reduction of dietary CP by 4.5% without deteriorating the accumulative growth performance and carcase traits of broilers.

HIGHLIGHTS

- Gly addition that provides 2.32% total Gly\textsuperscript{+}Ser allows dietary CP reduction of 4.5% without compromising the accumulative growth performance and carcase traits of broilers.
- Maintaining 2.32% total Gly\textsuperscript{+}Ser via Gly fortification could not completely overcome the depression of growth performance in broilers fed the diet with a 6% CP reduction.

Introduction

The excretion of nitrogen (N) originating from dietary protein is highly associated with the environmental issues arisen from intensive animal production (Morse 1995). One efficient way to limit N excretion of animals is to lower the dietary crude protein (CP) level. Moderate reduction of dietary CP level elicits no compromise of broiler performance, as long as the essential amino acids (EAA) are supplemented (Namroud et al. 2008; Belloir et al. 2017). However, several studies have shown that growth performance and carcase traits become inferior to those of birds fed the normal-CP diets when dietary CP level is decreased by more than three to four percentage points, even though all the EAA requirements are apparently met (Aletor et al. 2000; Bregendahl et al. 2002). When prepare low-CP diets, the reduced inclusion of oil due to the reduced inclusion of soybean meal may also impact broiler performance. A critical reason for the impaired performance of birds induced by low-CP
feeding could be the underestimation of glycine (Gly) (Namroud et al. 2008; Berres et al. 2010), which is a component of uric acid and protein structure, and serves as a precursor to multiple biological substances such as creatinine, glutathione, nucleic acid bases and bile acids (Corzo et al. 2004).

Gly is traditionally viewed as a limiting amino acids (AA) for chickens at an early stage, for which reason the Gly requirement has been estimated specifically for broilers during starter period instead of grower or the overall period (Dean et al. 2006; Ospina-Rojas et al. 2013). However, Gly is also classified as semi-essential for broilers within the grower period (Graber and Baker 1973; Ospina-Rojas et al. 2013), and can become limiting throughout the whole life of broilers as dietary CP reduced below a certain level (Awad et al. 2015; Belloir et al. 2017). On a molar basis, serine (Ser) is thought to be as effective as Gly for various functions in poultry because of the interconversion between them (Sugahara and Kandatsu 1976), which makes it necessary to consider the requirements of these AA jointly. It was indicated that dietary Gly + Ser level should be specifically considered for improving broiler performance when low-CP diets are formulated (Dean et al. 2006; Ospina-Rojas et al. 2013). However, the existing studies are inconsistent with respect to optimal dietary level of Gly + Ser for supporting broiler performance (Corzo et al. 2004; Ospina-Rojas et al. 2013; Awad et al. 2015). It is evident that the main factor causing differences among various estimates of Gly + Ser level is the reduction extent of dietary CP level (Waguespack et al. 2009; Ospina-Rojas et al. 2013; Awad et al. 2015). Through regression analysis, the requirement of total Gly + Ser level of broilers fed the diets with more than 3% CP reduction was estimated at 2.32% (Dean et al. 2006), however, the increased CP level in the diets accompanied by Gly addition was ignored. This might mislead the ability of Gly addition to allow dietary CP reduction to maintain a standard performance of broilers. Besides, little study was available to corroborate if maintaining an adequate level of dietary Gly + Ser could normalise the accumulative growth performance, carcase traits and the relative weight of immune organs of broilers fed the diet with a CP reduction by more than 3%. Thereby, the present study was conducted to explore broiler responses to low-CP diets fortified with Gly, validating if maintenance of adequate (2.32%) Gly + Ser in the diets with CP reduction by more than 3% could restore the growth performance, carcase traits, and the relative weight of immune organs along with serum biochemical parameters in broilers during the whole production period.

**Materials and methods**

**Animals and experimental design**

The experimental protocols and procedures were approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences. A crowd of 1-d-old male Arbour Acres broiler chicks were raised in three-tier battery cages in an environmentally controlled room, and acclimated to a commercial pre-starter diet and the environment during the first week. At 8 day of age, 1152 healthy birds with similar body weight were selected and divided into 8 groups, each of which fed with one of the following diets: (I) normal diet (containing 22% and 20% CP during starter period (8-21 d) and grower period (22–42 d), respectively; (II, III, IV, V) diets with 1.5%, 3%, 4.5% and 6% points less CP than normal diet, respectively; (VI, VII, VIII) diets with 3%, 4.5% and 6% points less CP than normal-CP diet, respectively, and supplemented with Gly to achieve 2.32% total Gly + Ser (Dean et al. 2006). All EAA contents were kept identical across groups by adding crystalline AA. Each group consisted of 12 cages with 12 birds each. All birds were raised in wired three-level battery cages (100 cm long × 80 cm wide × 40 cm high/cage). The lighting schedule was 23 h light and 1 h dark throughout the experiment. The room temperature was maintained at 34°C for the first week, and then reduced by 3°C per week until it reached 24°C. All birds had free access to the pelleted feed and water throughout the trial period. Prior to diet formulation, representative samples of corn and soybean meal were taken to determine their CP and AA contents, according to the method of Association of Official Analytical Chemists procedures (AOAC International 2006). The composition of starter diets (Table 1) and grower diets (Table 2) were based on the analytical values of CP and AA contents of corn and soybean meal. All diets were formulated to meet the AA requirements of broilers, which were determined by the AminoChick® 2.0 (Evonik Industrial Group, Germany), a software tool predicting optimum amino acids contents in poultry feed.

**Measurement of growth performance**

Bodyweight and feed intake were recorded for each replicate on d 21 and 42. Average body weight (ABW) on d 21 and 42, along with average daily gain (ADG),
average daily feed intake (ADFI), and feed conversion ratio (FCR) of birds during starter period (8–21 d) and the overall period (8–42 d) were calculated.

Measurement of carcass traits

At 21 and 42 d of age, two birds from each replicate were randomly selected and weighed after starvation for 12 h, followed by sacrifice via exsanguination. The carcases were plucked and eviscerated. The weights of eviscerated carcase, breast muscle, leg muscle and abdominal fat were then recorded separately. Afterwards, the eviscerated yield (EY, the percentage of eviscerated carcase weight to body weight), breast muscle yield (BMY, the percentage of breast muscle weight to eviscerated carcase weight), leg muscle yield (LMY, the percentage of leg muscle weight to eviscerated carcase weight), and abdominal fat percentage (AFP, the percentage of abdominal fat weight to eviscerated carcase weight) were calculated.

Determination of the relative weight of immune organs

Immediately upon the determination of carcase traits on d 21 and 42, the immune organs including spleen, thymus, and bursa of Fabricius of birds were removed and weighed for the determination of their relative weight, which was expressed as the ratio of organ weight (g) to body weight (kg).
Biochemical assay of serum samples

At 21 and 42 d of age, two birds from each replicate pen were randomly selected for blood collection from wing vein. Serum samples were obtained by centrifugation of blood at 3000 xg for 10 min at 4°C, followed by storage at -30°C until analysis. Serum total protein (TP), albumin, urea nitrogen (UN), uric acid (UA) and creatinine were all quantified colorimetrically using commercial assay kits from the Institute of Jiancheng Biological Engineering (Nanjing, China) with a VersaMax microplate reader (Molecular Devices, San Jose, USA).

Statistical analysis

Data were presented as mean ± standard deviation and were subjected to one-way ANOVA using the GLM procedure of SPSS 18.0 software after the assessment of normality and homoscedasticity. Differences among treatments were determined by Tukey's multiple range test. Besides, all data excluded those from normal CP and 1.5% points less CP groups were analysed as a 3 x 2 factorial arrangement. Significant differences among treatment means were determined at p < .05.

Results

Growth performance

Broilers fed a diet containing 1.5% points less CP had growth performance during starter and the overall periods that were not different (p > .05) from those fed the normal-CP diet (Table 3). Reducing dietary CP

Table 2. Composition and nutrient levels of the grower (22–42 d) diets (% as fed).

| Ingredients          | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 | Diet 7 | Diet 8 |
|----------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Gly+                 | 3.10   | 3.10   | 3.10   | 3.10   | 3.10   | 3.10   | 3.10   | 3.10   |
| Ca                   | 0.90   | 0.90   | 0.90   | 0.90   | 0.90   | 0.90   | 0.90   | 0.90   |
| Available P          | 0.40   | 0.40   | 0.40   | 0.40   | 0.40   | 0.40   | 0.40   | 0.40   |
| Lys                  | 0.99 (1.12) | 0.99 (1.12) | 0.99 (1.05) | 0.99 (1.04) | 0.99 (1.03) | 0.99 (1.11) | 0.99 (1.10) | 0.99 (1.06) |
| Met + Cys            | 0.75 (0.65) | 0.75 (0.66) | 0.75 (0.79) | 0.75 (0.80) | 0.75 (0.78) | 0.75 (0.84) | 0.75 (0.79) | 0.75 (0.77) |
| Trp                  | 0.19 (0.22) | 0.17 (0.19) | 0.16 (0.18) | 0.16 (0.17) | 0.16 (0.18) | 0.16 (0.17) | 0.16 (0.17) | 0.16 (0.18) |
| Thr                  | 0.65 (0.75) | 0.65 (0.76) | 0.65 (0.72) | 0.65 (0.71) | 0.65 (0.69) | 0.65 (0.72) | 0.65 (0.70) | 0.65 (0.68) |
| Gly + Ser            | 1.79 (1.81) | 1.63 (1.68) | 1.45 (1.46) | 1.22 (1.25) | 0.95 (0.96) | 2.32 (2.36) | 2.32 (2.35) | 2.32 (2.34) |
| dEB, mEq/kg          | 215    | 215    | 215    | 215    | 215    | 215    | 215    | 215    |

aSupplied the following per kg of diet: 12,500 IU of vitamin A, 4,480 IU of vitamin D3, 30 mg of vitamin E, 1.5 mg of vitamin B1, 6.0 mg of vitamin B2, 0.02 mg of Vitamin B12, 3.0 mg of Vitamin B6, 14 mg of calcium pantothenate, 80 mg of nicotinic acid, 0.10 mg of biotin, 1.5 mg of folic acid, and 3 mg of vitamin K3.

bSupplied the following per kg of diet: 100 mg of Fe, 12.5 mg of Cu, 95 mg of Zn, 88 mg of Mn, 0.9 mg of I, 0.11 mg of Se, and 150 mg of ethoxiquin.

cValues in parenthesis represent analysed contents of nutrients. As predicted by AminoChick® 2.0, the requirements of Lys, Met + Cys, Trp and Thr in the grower diet of broilers are 0.99%, 0.75%, 0.16%, 0.65%, all other essential amino acids (Ile, Leu, Val, Phe) along with Arg and His were apparently met.

dME: metabolisable energy.

deEB: dietary electrolyte balance.
by 3% had no influences (p > 0.05) on ABW, ADG or ADFI, in contrast to FCR (p < 0.05). When the CP level was further decreased by 4.5% or 6%, almost all performance parameters were impaired (p < 0.05). Adding Gly had beneficial effects (p < 0.05) on ABW, ADG and FCR of birds. Gly fortification in the diet with a 3% reduction of CP level decreased (p < 0.05) the FCR during starter and the overall periods to a level equal (p > 0.05) to that of normal-CP group. Furthermore, Gly fortification in the diet with a 4.5% reduction of CP level restored (p < 0.05) the FCR during starter period, accumulative ADG and ADFI, and along with the final ABW to the same (p > 0.05) levels as those in normal-CP group. Comparatively, Gly fortification in the diet with a 6% reduction of CP level induced an elevated (p < 0.05) ABW on d 21 and 42, concurrent with an increased (p < 0.05) ADG and a reduced (p < 0.05) FCR during starter and the overall periods, but which were still inferior (p < 0.05) to those in normal-CP group.

**Carcass traits and relative weight of immune organs**

There were no effects (p > 0.05) of dietary CP level reduction by 1.5% on carcass traits of broilers on d 21 and 42 (Table 4). Lowering dietary CP level by 3% elevated (p < 0.05) AFP of birds on d 42, while the reduction by 4.5% resulted in an increased (p < 0.05) AFP on both d 21 and 42. When the reduction of dietary CP level reached 6%, birds showed an increase (p < 0.05) in AFP with a reduction (p < 0.05) in BMI on both d 21 and 42. Gly fortification in the diet with a 4.5% CP reduction resulted in AFP of birds on both d 21 and 42 comparable with (p > 0.05) that of birds fed the normal-CP diet. Moreover, birds fed the low-CP (6% points less) diet with Gly fortification had a similar (p > 0.05) BMI on both d 21 and 42 relative to birds fed the normal-CP diet. As shown in Table 5, there were no differences (p > 0.05) in the relative weight of thymus, spleen and bursa of Fabricius of birds on either d 21 or 42 among groups. A reduced (p < 0.05) relative weight of spleen was observed in 6% points less CP group as compared with that in 3% points less CP group.

**Table 3. Effects of glycine (Gly) supplementation to low-crude protein (CP) diets on growth performance of broilers.**

| CP decrement | Gly | ABW, 21 d | ADG | ADFI | FCR | ABW, 42 d | ADG | ADFI | FCR |
|--------------|-----|-----------|-----|-----|-----|-----------|-----|-----|-----|
| Normal (0)   | –   | 862 ± 470a | 49.60 ± 3.30a | 73.10 ± 4.40a | 1.47 ± 0.07a | 2547 ± 127b | 65.10 ± 3.10a | 110.10 ± 5.20ab | 1.69 ± 0.06b |
| 1.500%       | –   | 864 ± 400a | 49.70 ± 3.00a | 74.50 ± 2.50a | 1.50 ± 0.08a | 2512 ± 144b | 64.20 ± 3.50a | 110.70 ± 6.20ab | 1.72 ± 0.03cd |
| 3%           | –   | 843 ± 230b | 47.60 ± 5.50b | 74.50 ± 2.40b | 1.56 ± 0.07b | 2483 ± 144b | 62.80 ± 3.20b | 111.40 ± 3.90b | 1.77 ± 0.07abc |
| 4.500%       | –   | 785 ± 330c | 43.80 ± 2.60c | 69.50 ± 4.10c | 1.58 ± 0.08c | 2392 ± 85c | 59.60 ± 4.20c | 106.50 ± 5.20c | 1.79 ± 0.06c  |
| 6%           | –   | 711 ± 370d | 38.30 ± 2.40d | 66.00 ± 2.30d | 1.72 ± 0.10d | 2079 ± 149d | 52.00 ± 4.70d | 100.70 ± 5.60d | 1.94 ± 0.15ab  |
| 3%           | +   | 837 ± 420b | 47.60 ± 2.40b | 72.10 ± 2.00b | 1.51 ± 0.07b | 2532 ± 133b | 63.70 ± 3.30b | 110.20 ± 5.70b | 1.73 ± 0.06bc  |
| 4.500%       | +   | 817 ± 480ab| 45.50 ± 3.10ab| 68.50 ± 3.60ab| 1.50 ± 0.06ab| 2440 ± 93ab | 62.00 ± 2.00ab | 107.80 ± 4.00ab | 1.74 ± 0.07abcd|
| 6%           | +   | 784 ± 300c | 42.70 ± 2.00c | 66.40 ± 2.60c | 1.56 ± 0.09c | 2275 ± 192c | 56.30 ± 4.00c | 101.70 ± 4.20c | 1.81 ± 0.12c   |

Main effects

| CP decrement | Gly | ABW, 21 d | ADG | ADFI | FCR |
|--------------|-----|-----------|-----|-----|-----|
| 3%           | –   | 840 ± 330a | 47.60 ± 2.00a | 73.30 ± 2.50a | 1.54 ± 0.07a |
| 4.500%       | –   | 801 ± 430b | 44.70 ± 3.00b | 69.00 ± 3.90b | 1.54 ± 0.07b |
| 6%           | –   | 747 ± 490c | 40.50 ± 3.10c | 66.20 ± 2.40c | 1.64 ± 0.12c |
| Gly          | –   | 780 ± 620b | 43.20 ± 4.40b | 70.00 ± 4.60b | 1.62 ± 0.10b |
|              | +   | 813 ± 450c | 45.20 ± 3.20c | 69.00 ± 3.08c | 1.52 ± 0.08c |

*p*-value

| CP decrement | Gly | ABW, 21 d | ADG | ADFI | FCR |
|--------------|-----|-----------|-----|-----|-----|
| 3%           | –   | 81 ± 620b  | 43.20 ± 4.40b | 70.00 ± 4.60b | 1.62 ± 0.10b |
| Gly          | –   | 780 ± 620b | 43.20 ± 4.40b | 70.00 ± 4.60b | 1.62 ± 0.10b |

**Serum biochemical parameters**

Lowering dietary CP level did not affect (p > 0.05) serum TP, albumin, UA and creatinine contents of broilers on d 21, as well as serum TP, UN and creatinine contents on d 42 (Tables 6 and 7). However, dietary CP reduction by 6% led to an increase (p < 0.05) in serum UN content on d 21, along with reductions in serum albumin and UA contents on d 42. Adding Gly plays a role in lowering (p < 0.05) serum UN content of birds on d 21. Gly fortification in the diet with a 4.5% CP reduction lowered (p < 0.05) serum UN content on d 21, while Gly fortification in the diet containing 6% CP restoration (p < 0.05) serum UN content on d 42 to the same levels as birds fed the normal-CP diet.
Main effects

Large effects

Normal (0) – 72.91 ± 1.51 22.67 ± 1.266 18.07 ± 1.76 1.19 ± 0.287 72.44 ± 1.37 27.79 ± 1.977 18.77 ± 1.69 1.97 ± 0.307 4.500% – 72.95 ± 2.58 21.34 ± 1.514 16.97 ± 1.00 1.71 ± 0.364 72.65 ± 2.29 26.37 ± 1.494 19.51 ± 1.07 2.55 ± 0.394 3% – 72.31 ± 2.48 20.84 ± 1.33b 17.37 ± 1.76 1.77 ± 0.31 71.27 ± 3.60 24.69 ± 1.30 19.06 ± 2.95 2.72 ± 0.48 4.500% + 72.65 ± 2.28 22.31 ± 1.73c 17.54 ± 1.35 1.54 ± 0.27c 73.26 ± 1.27 26.53 ± 1.69c 19.06 ± 0.71 2.38 ± 0.56c

Discussion

Without Gly addition, lowering dietary CP by 1.5% had no adverse impacts on broiler performance. However, the feed efficiency of broilers was worsened due to dietary CP reduction by 3%, while almost all performance parameters of birds were impaired by lowering dietary CP level by 4.5% and 6%, which were similar to previous studies (Namroud et al. 2008; Ospina-Rojas et al. 2013). The reason for these detrimental effects could be associated with the roles of Gly (or Ser), being a key limiting AA below a certain level of dietary CP (Awad et al. 2015). There were controversies surrounded in the importance of Gly addition that achieved high Gly + Ser level in diets for supporting performance of broilers (Waguespack et al. 2009; Ospina-Rojas et al. 2013; Awad et al. 2015). It is
possible that the requirement of Gly + Ser of broilers depends on the reduction extent of dietary CP level. The present study validated that Gly addition that provided 2.32% total Gly + Ser in the diet with a 3% reduction of CP level normalised the growth performance of birds. Besides, when Gly fortification in the diet with a 4.5% CP reduction, FCR during starter period, final ABW, along with the accumulative ADG and FCR were also restored to standard levels. However, Gly addition failed to completely overcome the adverse effects of dietary CP reduction by 6% on bird performance. These findings confirmed that maintaining a total Gly + Ser level of 2.32% in diets via Gly addition allowed to lower dietary CP level by 4.5% without compromising the accumulative growth performance of birds. Interestingly, Gly fortification in low-CP diets did not alter ADFI, suggesting that the positive effect of Gly addition on broiler growth

Table 6. Effects of glycine (Gly) supplementation to low-crude protein (CP) diets on serum biochemical parameters of broilers at 21 d of age.

| CP decrement | Gly | TP (mg/mL) | ALB (mg/mL) | UN (mmol/L) | UA (mmol/L) | CRE (µmol/L) |
|--------------|-----|------------|-------------|-------------|-------------|--------------|
| Normal (0)   | –   | 30.88 ± 2.98 | 15.21 ± 1.74 | 0.78 ± 0.15 | 531.33 ± 263.32 | 44.13 ± 6.85 |
| 1.500%       | –   | 29.15 ± 3.72 | 13.91 ± 1.31 | 0.64 ± 0.09 | 495.64 ± 209.01 | 39.20 ± 6.16 |
| 3%           | –   | 29.48 ± 4.30 | 14.02 ± 1.93 | 0.72 ± 0.16 | 561.08 ± 246.30 | 41.49 ± 8.27 |
| 4.500%       | –   | 27.43 ± 4.11 | 13.81 ± 2.84 | 0.81 ± 0.07 | 482.08 ± 246.27 | 38.72 ± 6.71 |
| 6%           | –   | 29.26 ± 3.93 | 14.48 ± 1.94 | 0.70 ± 0.14 | 448.33 ± 208.07 | 39.26 ± 9.48 |
| 4.500%       | +   | 29.95 ± 5.56 | 13.34 ± 2.50 | 0.63 ± 0.20 | 539.67 ± 290.44 | 42.51 ± 8.18 |
| 6%           | +   | 28.98 ± 3.59 | 13.79 ± 1.55 | 0.72 ± 0.13 | 497.08 ± 221.90 | 38.94 ± 8.27 |

Main effects

| CP decrement | Gly | TP (mg/mL) | ALB (mg/mL) | UN (mmol/L) | UA (mmol/L) | CRE (µmol/L) |
|--------------|-----|------------|-------------|-------------|-------------|--------------|
| 3%           | –   | 29.37 ± 4.03 | 14.25 ± 1.91 | 0.71 ± 0.15 | 589.58 ± 235.65 | 40.05 ± 7.34 |
| 4.500%       | –   | 28.69 ± 5.47 | 13.57 ± 2.63 | 0.72 ± 0.17 | 510.88 ± 264.98 | 40.61 ± 7.57 |
| 6%           | –   | 28.80 ± 3.78 | 13.73 ± 1.77 | 0.81 ± 0.19 | 472.71 ± 211.84 | 39.10 ± 8.70 |
| 4.500%       | +   | 29.51 ± 4.55 | 13.83 ± 2.25 | 0.70 ± 0.14 | 618.08 ± 231.67 | 38.60 ± 6.32 |
| 6%           | +   | 29.39 ± 4.33 | 13.87 ± 2.03 | 0.68 ± 0.09 | 551.61 ± 247.87 | 40.02 ± 7.63 |

p-value

| CP decrement | Gly | TP (mg/mL) | ALB (mg/mL) | UN (mmol/L) | UA (mmol/L) | CRE (µmol/L) |
|--------------|-----|------------|-------------|-------------|-------------|--------------|
| 3%           | –   | .86        | .54         | .04         | .24         | .80          |
| 4.500%       | –   | .41        | .94         | .001        | .34         | .92          |
| 6%           | –   | .54        | .76         | .09         | .99         | .34          |

Table 7. Effects of glycine (Gly) supplementation to low-crude protein (CP) diets on serum biochemical parameters of broilers at 42 d of age.

| CP decrement | Gly | TP (mg/mL) | ALB (mg/mL) | UN (mmol/L) | UA (mmol/L) | CRE (µmol/L) |
|--------------|-----|------------|-------------|-------------|-------------|--------------|
| Normal (0)   | –   | 39.25 ± 6.43 | 15.32 ± 1.93 | 0.50 ± 0.23 | 275.50 ± 69.51 | 49.03 ± 11.04 |
| 1.500%       | –   | 40.18 ± 7.65 | 14.60 ± 1.85 | 0.44 ± 0.14 | 284.17 ± 75.03 | 40.61 ± 7.57 |
| 3%           | –   | 37.16 ± 4.91 | 14.47 ± 1.54 | 0.42 ± 0.24 | 242.08 ± 95.15 | 44.63 ± 7.40 |
| 4.500%       | –   | 37.20 ± 3.56 | 14.79 ± 1.10 | 0.50 ± 0.20 | 170.83 ± 41.93 | 44.50 ± 7.48 |
| 6%           | –   | 33.29 ± 7.11 | 12.61 ± 2.85 | 0.51 ± 0.19 | 113.67 ± 55.80 | 42.16 ± 5.95 |
| 3%           | +   | 35.64 ± 3.81 | 14.23 ± 1.91 | 0.39 ± 0.09 | 223.08 ± 57.93 | 44.28 ± 3.33 |
| 4.500%       | +   | 38.89 ± 5.54 | 14.89 ± 1.56 | 0.40 ± 0.07 | 189.83 ± 77.05 | 44.81 ± 6.78 |
| 6%           | +   | 36.05 ± 3.31 | 14.55 ± 1.34 | 0.54 ± 0.19 | 140.17 ± 32.41 | 44.71 ± 7.24 |

Main effects

| CP decrement | Gly | TP (mg/mL) | ALB (mg/mL) | UN (mmol/L) | UA (mmol/L) | CRE (µmol/L) |
|--------------|-----|------------|-------------|-------------|-------------|--------------|
| 3%           | –   | 36.40 ± 4.37 | 14.35 ± 1.71 | 0.40 ± 0.18 | 232.58 ± 77.65 | 44.45 ± 5.61 |
| 4.500%       | –   | 38.04 ± 4.63 | 14.84 ± 1.32 | 0.45 ± 0.15 | 180.33 ± 61.49 | 44.65 ± 6.98 |
| 6%           | –   | 34.67 ± 5.61 | 13.58 ± 2.39 | 0.53 ± 0.19 | 126.92 ± 46.64 | 43.43 ± 6.61 |
| 3%           | +   | 35.88 ± 5.56 | 13.95 ± 2.15 | 0.48 ± 0.21 | 175.53 ± 84.94 | 43.76 ± 6.87 |
| 4.500%       | +   | 36.86 ± 4.45 | 14.55 ± 1.60 | 0.44 ± 0.14 | 184.36 ± 66.67 | 44.60 ± 5.87 |

p-value

| CP decrement | Gly | TP (mg/mL) | ALB (mg/mL) | UN (mmol/L) | UA (mmol/L) | CRE (µmol/L) |
|--------------|-----|------------|-------------|-------------|-------------|--------------|
| .06          | .06  | .06        | .06         | <.001       | .79         | .59          |
| .40          | .16  | .46        | .56         | .59         | .72         | .72          |

Means with no common superscripts within the column of each classification are significantly (p < .05) different. 
1TP: total protein (mg/mL); ALB: albumen (mg/mL); UN: urea nitrogen (mmol/L); UA: uric acid (µmol/L); CRE: creatine (µmol/L).
2The normal CP level in the starter (8–21 d) diet and grower diet (22–42 d) was 22% and 20%, respectively.
3Data were analysed as a 3 × 2 factorial arrangement, excluding normal CP and 1.5% points less CP groups.
performance was not related to the increased feed intake, instead, it might be attributed to the roles of Gly in enterocyte development or mucin secretion, benefiting nutrient utilisation and protein synthesis (Ospina-Rojas et al. 2013; Wang et al. 2014).

Carcase traits are particularly important for broiler production benefit. The present study showed that dietary CP reduction by 6% lowered BMY of birds on d 21 and 42, while the reduction by 4.5% and 6% led to an increase in AFP on d 21 and 42, implying that the carcase traits became inferior to those of birds fed the normal-CP diet when dietary CP level is decreased by 4.5% or higher. This was basically consistent with previous studies (Awad et al. 2017; Belloir et al. 2017). Notably, Gly fortification in the diet with a 4.5% reduction of CP resulted in a similar AFP of birds as compared with those fed the normal-CP diet. This might be due to the ideal AA profile in this low-CP diet fortified with Gly, resulting in an increase of energy needed for N deposition, thus leaving less energy to be deposited in abdomen (Smith et al. 1999). When Gly fortification in the diet with a 6% reduction of CP level, birds had a similar BMY to those fed the normal-CP diet. This might highlight the importance of Gly in maintaining BMY of birds fed this low-CP diet, presumably because of the role of Gly as a precursor for the synthesis of creatine (Ngo et al. 1977), which is the main energy source for muscle and can improve nutrient utilisation for muscle development (Allen 2012). Measurement of internal immune organ weight is a common method of estimating immune status in chickens (Wu et al. 2018). Similar to the study of Ndazigaruye et al. (2019), the current study revealed that dietary CP reduction did not affect the relative weight of immune organs in broilers. Little study was available regarding the effect of Gly addition on the relative weight of immune organs of chickens. Herein, the absence of modification of the relative weight of immune organs including thymus, spleen and bursa of Fabricius implied that Gly fortification in low-CP diets elicited no benefit for the immune function of broilers.

Serum biochemical indices are often used to monitor health and also to reflect the nutritional status of chickens. It was reported that lowering dietary CP level exerted no influences on serum TP and albumin contents in animals (Ndazigaruye et al. 2019). Whereas Law et al. (2018) and Ospina-Rojas et al. (2012) observed reductions of serum TP and albumin contents in broilers with dietary CP reduction by three to four percents. Herein, dietary CP reduction did not change serum TP and albumin contents in birds on d 21, while the reduction of dietary CP by 6% triggered a reduction of serum albumin content of birds on d 42, which might be owe to the deficit of AA ingested by birds when dietary CP was sharply reduced without Gly addition (Corzo et al. 2009). Gly fortification in the diet with a 6% CP reduction restored the decreased albumin content on d 42 to a standard level, indicating that Gly addition might increase AA intake, favouring albumin synthesis of birds at the late stage. Blood UN and UA contents could be used as influential criteria to reflect N utilisation in animals (Donsbough et al. 2010). In this study, serum UN content of birds on d 21 remained unchanged in response to dietary CP reduction by 4.5% or less, but it was elevated with dietary CP reduction by 6%. This might be responsible by the short of some nonessential AA of birds fed a diet with a 6% CP reduction, resulting in an imbalanced AA profile with a subsequent deficiency of N utilisation (Nyachoti et al. 2006; Heo et al. 2008). When fortified with Gly, the reduced serum UN of birds fed a diet containing 6% points less CP was restored to a standard level, presumptively because that Gly addition spared several nonessential AA and in turn improved N utilisation (Taylor et al. 1981; Ospina-Rojas et al. 2012). Alternatively, Gly fortification reduced urea production by inhibiting arginase activity (Austic and Nesheim 1970). Similar to the previous studies (Powell et al. 2009; Awad et al. 2017; Law et al. 2018), we observed a reduction of serum UA content on d 42 with dietary CP reduction by 4.5% or 6%, which could be related to the lower ingestion with the subsequent reduced catabolism of AA (Awad et al. 2017; Law et al. 2018). Although Gly is directly involved in the synthesis of UA, we did not find an alteration of serum UA content in response to Gly fortification in the low-CP diets, which did not agree with the study of Awad et al. (2017). The discrepancy might be associated with the difference in total Gly + Ser level in diets. Creatinine, a final product of the degradation of creatine and phosphocreatine in skeletal muscle, can diffuse into bloodstream and can be then excreted in urine (Nelson and Cox 2000). In this study, neither dietary CP reduction nor Gly fortification exerted an effect on serum creatinine content, which might be responsible by that non-enzymatic formation of creatinine from creatine is approximately constant (Wyss and Kaddurah-Daouk 2000).

Conclusions

Dietary CP reduction by 1.5% did not affect broiler performance. However, dietary CP reduction by 3% resulted in a declined feed efficiency of broilers, while
birds fed the diet with a 4.5% or more CP reduction had deteriorations in both growth performance and carcase traits as compared to those received the normal-CP diet, despite meeting the requirements of EAA. Maintaining Gly + Ser level at 2.32% via Gly fortification allowed for 4.5% reduction of dietary CP level without undermining the accumulative growth performance and carcase traits of broilers. Comparatively, Gly fortification that provided 2.32% Gly + Ser level in diet could not fully recover the depressed performance of broilers fed the diet with a 6% CP reduction.

Disclosure statement
The authors declare that they have no conflicts of interest associated with this manuscript.

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