Effect of glycine equivalent levels in low protein diet containing different SID threonine concentrations on performance, serum metabolites and muscle creatine of broiler chickens

Paschal C. Aguihe, Kazuo A. Hirata, Camilo I. Ospina-Rojas, Tatiana C. dos Santos, Paulo C. Pozza, Eustace A. Iyayi, and Alice E. Murakami

Department of Animal Production and Health Technology, Federal College of Wildlife Management, New Bussa, Nigeria; Department of Animal Science, Universidade Estadual de Maringá, Maringá, Brazil; CJ Corporation, Av. Engenheiro Luís Carlos Berrini, Monções, São Paulo, Brazil; Department of Animal Science, University of Ibadan, Ibadan, Nigeria

ABSTRACT
This study was conducted to evaluate glycine equivalent (Glyequi) requirement in low crude protein (CP) diets with different levels of standardised ileal digestible (SID) threonine (Thr) on performance, serum metabolites, and muscle creatine of broiler chickens from 1 to 21 d age. A total of 1275 one-day-old male Cobb-Vantress chicks were distributed in a completely randomised factorial arrangement for a total of 15 treatments with 5 replicates of 17 birds each. Diets were formulated to comprise three dietary SID of Thr concentrations 6.9, 8.1 and 9.3 g/kg and five dietary Glyequi levels (12.8, 14.3, 15.8, 17.3 and 18.8 g/kg). An interaction (p < 0.05) was observed between the Glyequi and SID Thr levels for feed:gain and serum uric acid (SUA). With increasing levels of Glyequi, feed:gain decreased linearly (p < 0.05) in 6.9 and 8.1 g/kg SID Thr diets while SUA decreased linearly (p < 0.05) in 6.9 g/kg SID Thr diet. In diet containing 9.3 g/kg SID Thr, increasing Glyequi level optimised feed:gain (p = 0.04) at an estimated minimum level of 15.5 g/kg. Glyequi levels resulted in a quadratic effect (P < 0.05) on BWG, feed:gain, muscle creatine with optimum point of 17.2, 16.7, 17.1 g/kg, respectively. Therefore, minimum requirement of Glyequi in low CP (174.0 g/kg) diet needed to optimised performance is 15.5 g/kg in 9.3 g/kg SID Thr diet, but could be or higher than 20.9 g/kg in 6.9 g/kg and 8.1 g/kg SID Thr diets for broilers.

HIGHLIGHTS
• Feeding a low-protein diet of 174 g/kg with adequate Thr concentration at higher Glyequi levels could support performance in broilers of 1–21 d old.
• Provision of 15.5 g/kg Glyequi in low CP diet containing 9.3 g/kg SID Thr level is required to improve performance.
• Sufficient supply of dietary Thr concentration represents a sparing effect of Gly especially at marginal levels of dietary Glyequi in low-CP diets.

Introduction
A major concern of modern poultry industry is to reduce feed cost for optimal economic returns and environmental pollution caused by nitrogen excretion and also ammonia emissions. Crude protein (CP) is one of the major cost components of poultry diets that receive thoughtful considerations during feed formulation (Najafi et al. 2017). In poultry production, the utilisation of low-CP diets with adequate amino acid (AA) supplementation has become a powerful strategy to lessen production cost, nitrogen discharge, and ammonia outflows (Roberts et al. 2007; Belloir et al. 2017). Dietary CP level could possibly be reduced if there were adequate minimum levels of amino acids needed to support growth of broilers. However, previous workers have reported that feeding a low CP diet had resulted to poor growth performance in poultry, despite meeting the requirements of all essential amino acids (Hussein et al. 2001; Namroud et al. 2008; Awad et al. 2014; Yang et al. 2015). The potential endearing explanation of decreased growth of growing birds fed low CP diets is inadequate provision of...
The specific AAs notably Gly (Aleto et al. 2000; Dean et al. 2006; Waguespack et al. 2009; Awad et al. 2015; Wang et al. 2020). Reducing CP concentration of plant-based diets causes a substantial drop in dietary Gly levels as low CP diet entails a great reduction in intact protein concentration (Dean et al. 2006; Ospina-Rojas et al. 2012). Along these lines, it is conceivable that negligible concentrations of dietary Gly and underestimation of its requirements were observed to inhibit the growth efficiency in broiler chickens fed low CP diets, developed without animal by-products, since the amount of endogenous synthesis could not sustain optimal performance at the early stage (Yuan et al. 2012; Belloor et al. 2017; Wang et al. 2020). The maintenance requirement for Gly is relatively high such that the Gly synthesis may not be adequate to cover increased endogenous losses, peculiar requirements for protein accretion (Heger 2003; Wang et al. 2020). In formulation of broiler diet, dietary Gly is generally considered together with serine (Ser), due to their unrestricted metabolic inter-conversion (Velišek and Čejpek 2011). Various experiments have demonstrated that dietary Ser performs similar roles as dietary Gly provided it is supply in equimolar quantities (Sugahara and Kandatsu 1976). Thus, it has been recommended that dietary glycine equivalent (Glyequi) be calculated as the sum of the Gly concentration and the molar equivalent of Ser being 0.7143, to evaluate the effect of dietary Gly and Ser in poultry (Dean et al. 2006). Several studies have shown that keeping an adequate concentration of dietary Glyequi in poultry fed low CP diet, during the early weeks of age, could restore their accumulative performance (Dean et al. 2006; Siegert et al. 2015; Awad et al. 2017; Hofmann et al. 2019; Chrystal et al. 2020).

Threonine (Thr) is the third most limiting AA, especially in a low CP diet (Rezaeipour et al. 2012). In addition to the significant role of Thr as an essential AA in protein synthesis and component of the feather protein and gastrointestinal epithelium, as well as immunoglobulin molecules (Ojano-Dirain and Cejpek 2011). Various experiments have demonstrated that dietary Ser performs similar roles as dietary Gly provided it is supply in equimolar quantities (Sugahara and Kandatsu 1976). Thus, it has been recommended that dietary glycine equivalent (Glyequi) be calculated as the sum of the Gly concentration and the molar equivalent of Ser being 0.7143, to evaluate the effect of dietary Gly and Ser in poultry (Dean et al. 2006). Several studies have shown that keeping an adequate concentration of dietary Glyequi in poultry fed low CP diet, during the early weeks of age, could restore their accumulative performance (Dean et al. 2006; Siegert et al. 2015; Awad et al. 2017; Hofmann et al. 2019; Chrystal et al. 2020).

Threonine (Thr) is the third most limiting AA, especially in a low CP diet (Rezaeipour et al. 2012). In addition to the significant role of Thr as an essential AA in protein synthesis and component of the feather protein and gastrointestinal epithelium, as well as immunoglobulin molecules (Ojano-Dirain and Waldroup 2002; Sá et al. 2007), it is a precursor of Gly and Ser in metabolism (Baker et al. 1972; Lee et al. 2011; Neto et al. 2012). Dietary Thr levels higher than the required may decrease the need for dietary Gly (Ospina-Rojas et al. 2013a). Adequate Thr supplementation has shown a sparing effect on Gly, especially at marginal levels of dietary Glyequi probably via a one-step reaction involving the enzymatic cleavage (Thr aldolase) of Thr to Gly and acetaldehyde (Rangel-Lugo et al. 1994, Yuan and Austic 2001; Ciftci and Ceylan 2004). On the other hand, adequate Glyequi supplementation can decrease the degradation of Thr to Gly by reducing the bioactivity of the liver Thr aldolase and dehydrogenase in birds (Bernardino et al. 2011). Thus, the de novo synthesis of Gly from Thr might partly cover the metabolic requirements for Gly through the Thr aldolase and dehydrogenase pathway (Le Floc’h et al. 1994). Therefore, the present study was conducted to evaluate the optimum dietary Glyequi needs of broiler chickens from 1 to 21 d of age in a low-CP diet containing different standardised ileal digestible (SID) Thr levels.

**Material and methods**

**Experimental facility and bird husbandry**

All experimental procedures involving the handling of live animals were carried out according to the guidelines of Ethics Committee for humane care and use of animals in research of the Universidade Estadual de Maringa, Brazil (Protocol no: CEUA 7501230419). A total number of 1-day old Cobb-Vantress male broilers (1,275) with similar body weight (46.4 g) were obtained from a commercial hatchery after being vaccinated against Marek’s disease and distributed across 75 deep litter pens (17 birds/pen). Each pen (2.00 × 1.00 m) had fresh rice husk as litter material and was equipped with 1 tube feeder and 3 nipple drinkers. The lighting schedule was 23 h light and 1 h darkness throughout the period of the experiment. The room temperature was maintained at 32°C for the first week and was decreased gradually until it reached 21°C to ensure the environmental comfort by using a thermostatically controlled heater, fans, and foggers. All birds were given ad-libitum access to feed in mash form and clean water throughout the trial period. The experimental birds and housing facilities were inspected twice daily, in the morning and the afternoon, thereby checking for mortality, general health status, availability of feed and water, as well as lighting, temperature and ventilation, and the occurrence of unexpected events.

**Experimental design and diets**

A 3 × 5 completely randomised factorial arrangement of treatments was adopted, which consisted of 3 varying concentrations of SID Thr (6.9, 8.1 and 9.3 g/kg, corresponding to 85, 100 and 115% of the recommended SID Thr (Rostagno et al. 2011), respectively) in combination with 5 levels of Glyequi (12.8, 14.3, 15.8, 17.3 and 18.8 g/kg). Each treatment had 5 replicates of 17 birds each. A basal diet with low CP concentrations (174 g/kg CP) and 12.45 MJ/kg ME was based on corn...
and soybean meal used as intact protein sources and formulated according to the analysed nutrient composition values and the nutritional recommendations for male broilers of average performance, as proposed by Rostagno et al. (2011), except for the Glyequi and SID Gly requirements (Table 1). The other experimental diets were obtained by supplementing the basal diet with Gly and L-Thr as replacements for the inert filler. Dietary levels of Glyequi were designed considering the SID Gly + Ser requirements for broilers according to Rostagno et al. (2011) and calculated as the sum of the Gly concentration and the molar equivalent of Ser being 0.7143, to evaluate the effect of dietary Gly and Ser in poultry (Dean et al. 2006). The basal diet was designed to be deficient in Glyequi and Thr levels. The other experimental diets were obtained by supplementing the basal diet with Gly and L-Thr to meet and exceed animals' requirements (dose-response). The SID AA values of the basal diets were calculated by using digestibility coefficients of corn and soybean meal (Rostagno et al. 2011). Representative samples of the experimental basal diet were taken for the analysis of CP and total AA compositions using standard laboratory procedures as described by AOAC International (2006). The nitrogen content of the sample was determined by Kjeldahl method and crude protein (CP) equivalent was calculated as $N$ (%) × 6.25. The analysed concentrations of CP and AA of the experimental basal diet were close to its calculated concentrations (Table 1).

**Growth performance**

The whole body weights of broilers in each replicate were weighed using a digital weighing balance at day 1 and thereafter on a weekly basis. A measured amount of feed was provided to all broiler chickens in each replicate and at the end of each week, feed refusals were weighed to calculate the weekly feed intake on replicate basis. Broiler mortality was recorded twice daily, and the weight of dead birds was used to adjust the feed intake per replicate. The data on feed intake and weight gain, corrected for mortality, were used to determine feed:gain as feed consumed (g) divided by weight gained (g) by the chicken.

**Blood serum evaluation**

At 21 d of age, two birds in each replicate pen were selected based on the average weight (± 5%) and blood samples (2mL/bird) were collected from the jugular vein. The samples were kept on ice and centrifuged at 3000 g for 10 minutes. The serum collected by replicate pen was stored at −70°C until used for analysis. The frozen serum samples were thawed at 4°C and serum concentrations of total protein.
albumin, uric acid, glucose, triglyceride and creatinine were measured using commercial reagent kits (Gold Analisa Ltda, Belo Horizonte, Minas Gerais, Brazil) according to procedures described by Tietz (1986).

**Measurements of relative weight of breast, liver, pancreas and muscle creatine**

On the last day of the experiment (21 d), another 2 birds per pen of similar weight close to the average pen weight were electrically stunned and exsanguinated. The weight of the entire defeathered carcase was recorded; the breast, liver and pancreas were removed, and their relative weights were calculated as the percentage of the live weight. Thereafter, pectoral muscles (without the keel bone) were removed to calculate their yield relative to live body weight and creatine concentration in the pectoral muscle was determined following the method described by Rose et al. (1927) and modified by Chamruspollert et al. (2002). In brief, using a meat grinder, the pectoral breast muscles were ground and 1g of ground meat was taken and put inside a test tube (25/150 mm) with 20 mL of 1 M sulphuric acid. Samples were subsequently autoclaved for 45 minutes at 1.1 kg/cm² pressure, and kept at 4°C pending analysis. The samples were further taken into 100 mL volumetric flasks. Tubes and samples were rinsed with deionised water twice for removal of all contents. 18 mL of 2M NaOH were added to each volumetric flask accompanied by 5 mL of sodium tungstate (100 g/kg) addition. At the same period, the overall volume of deionised water in the flasks was reduced to 100 mL. Thereafter, samples were rattled and then permitted to stand for 5 minutes before filtering. Subsequently, 10 mL of the clear filtrate was poured into another 100 mL graduated flask and thereafter, 5 mL of alkaline picrate and 10 mL of NaOH (100 g/kg) were introduced to convert creatine to creatinine. The samples were combined and made to remain for 10 minutes, and then added with deionised water to make up a final volume of 100 mL. Creatinine (Gold Analisa Ltda, Belo Horizonte, Minas Gerais, Brazil) was used as a standard solution (500 mg/mL), and the sample absorbance and standard were determined using spectrophotometer (Semi-auto Biochemistry Analyser, System BIO-2000, São Paulo, Brazil) at a 450 nm wavelength.

**Statistical analysis**

The study followed a completely randomised design of 5 × 3 factorial arrangements of treatments. Percentage data for mortality were transformed to arcsine square-root percentages for analysis. All data were analysed by the GLM procedure of SAS Institute (2012) to assess the effects of the dietary concentrations of SID Thr, Glyequi and the interaction between the SID Thr and Glyequi by the following model:

\[
Y_{ijk} = \mu + G_i + M_j + GM_{ij} + e_{ijk}
\]

where \(Y_{ijk}\) is the response variable, \(\mu\) is the common mean; \(G_i\) is the effect of the ith Glyequi, \(M_j\) is the effect of the jth Thr, \(GM_{ij}\) is the effect of the interaction of the ith Glyequi with the jth Thr; and \(e_{ijk}\) is error term.

When the ANOVA indicated significant treatment effects, means were separated using Tukey’s multiple range tests by LSMEANS procedure of SAS Institute (2012). Statistical differences were considered to be significant at \(P < 0.05\) unless otherwise stated. A polynomial regression model was applied and where quadratic responses \((P < 0.05)\) were detected, the optimal Glyequi level was calculated by taking the first derivative of the quadratic equation:

\[
\frac{dy}{dx} = b - 2cX
\]

where, \(X = \text{Glyequi}\) levels, \(b\) and \(c\) represent linear or quadratic regression coefficient.

**Results and discussion**

No interaction \((P > 0.05)\) was observed between SID Thr and Glyequi levels on BWG and feed intake at 21 d of age, however, an interaction \((P < 0.04)\) was recorded for feed:gain (Table 2). The feed:gain decreased linearly in response to increasing levels of Glyequi from 12.8 to 18.8 g/kg in birds fed both diets containing 6.9 \((P = 0.04; R^2 = 0.96)\) and 8.1 \((P = 0.03; R^2 = 0.93)\) g/kg SID Thr concentrations. A quadratic effect \((P = 0.04; R^2 = 0.97)\) for feed:gain in response to increasing levels of Glyequi was observed in birds fed 9.3 g/kg SID Thr with a low feed:gain ratio estimated at 15.5 g/kg Glyequi. As for interaction effects between Glyequi and SID Thr levels (Table 3) shows that feed:gain of birds offered 6.9 and 8.1 g/kg SID Thr diets at 18.8 g/kg Glyequi levels were decreased compared to 12.8 g/kg Glyequi. At higher concentration of SID Thr (9.3 g/kg), birds fed diet containing 15.8 g/kg Glyequi showed decreased feed:gain than their counterparts offered diet with 18.8 g/kg Glyequi. On the other hand, the result displayed in Table 3 shows that evaluating the different concentrations of Glyequi across the increasing dietary levels of SID Thr affected \((P < 0.05)\) feed:gain of birds when fed 17.3 and 18.8 g/kg Glyequi.
Table 2. Performance response of broilers on low protein diet (LPD) containing different dietary Glycine equivalent (Glyequi) level with varying concentrations of Standardized Ileal Digestible threonine (SID Thr) (1–21 d).

| SID Thr (g/kg) | Weight gain (g) | Feed intake (g) | Feed: gain |
|---------------|----------------|----------------|-----------|
| Glyequi (g/kg) |                |                |           |
| 12.8          | 6.9            | 773.7          | 1160.5    | 1.50     |
| 14.3          | 6.9            | 826.1          | 1215.7    | 1.47     |
| 15.8          | 6.9            | 837.1          | 1214.3    | 1.47     |
| 17.3          | 6.9            | 849.1          | 1226.3    | 1.44     |
| 18.8          | 6.9            | 858.8          | 1225.5    | 1.43     |
| 12.8          | 8.1            | 777.2          | 1120.1    | 1.44     |
| 14.3          | 8.1            | 836.6          | 1184.4    | 1.42     |
| 15.8          | 8.1            | 847.1          | 1176.0    | 1.39     |
| 17.3          | 8.1            | 853.8          | 1179.0    | 1.38     |
| 18.8          | 8.1            | 855.4          | 1172.1    | 1.37     |
| 12.8          | 9.3            | 803.2          | 1204.8    | 1.49     |
| 14.3          | 9.3            | 823.2          | 1209.6    | 1.47     |
| 15.8          | 9.3            | 836.9          | 1211.7    | 1.45     |
| 17.3          | 9.3            | 823.5          | 1205.4    | 1.47     |
| 18.8          | 9.3            | 811.8          | 1228.2    | 1.52     |
| SEM           |                | 20.38          | 32.89     | 0.03     |

Table 3. Interaction effects of Glycine equivalent (Glyequi) and Standardized Ileal Digestible threonine (SID Thr) levels on feed:gain* (1–21 d).

| SID Thr (g/kg) | Glyequi (g/kg) |
|---------------|----------------|
| 6.9           | 1.500ab       |
| 8.1           | 1.444ab       |
| 9.3           | 1.499ab       |
| 12.8          | 1.500ab       |
| 14.3          | 1.471ab       |
| 15.8          | 1.452ab       |
| 17.3          | 1.465ab       |
| 18.8          | 1.465ab       |

**Means in rows followed by different lowercase superscripts are statistically different (P < 0.05).
**Means in columns followed by different uppercase superscripts are statistically different (P < 0.05).

at or higher than the maximum level of 18.8 g/kg Glyequi evaluated in the present study, since there is no evidence of a plateau. This observation supports the claims that birds fed low CP diet with low or adequate level of SID Thr requires higher Glyequi requirement during the starting phase in order to maximise feed:gain (Ospina-Rojas et al. 2013a; Lambert et al. 2015). In addition, according to Ospina-Rojas et al. (2013b), Gly supplementation indirectly improves the integrity of the gastrointestinal tract by preventing Thr degradation and minimising the endogenous conversion of Ser to Gly, thus increasing the availability of these AAs for intestinal mucosa maintenance. However, in diet with 9.3 g/kg SID Thr, a quadratic response (P < 0.05) on feed:gain was recorded with increasing dietary Glyequi level demonstrating an optimum point estimated at 15.5 g/kg (Table 2). This is in accordance with the affirmation of previous studies which demonstrated that broilers fed low CP diets with suboptimal levels of dietary Glyequi required higher concentration of Thr to avoid any decrease in performance since Thr is a possible precursor of Gly synthesis (Corzo 2012; Ospina-Rojas et al. 2013a; Ospina-Rojas et al. 2013b; Siegert and Rodehutscord 2015). According to Ospina-Rojas et al. (2013b), sparing of Gly by Thr supplementation was more pronounced when dietary Thr was higher than required. The present study has shown that in order to optimised feed:gain in broilers fed low CP diet with SID Thr level of not more than 8.1 g/kg, there is need for higher requirement of Glyequi not less than 20.6 g/kg, whereas the Glyequi requirement reduced to 15.5 g/kg when the diet is in excess of the required SID Thr of 9.3 g/kg. This could be related to the metabolic conversion of excess Thr into Gly by enzymes Thr aldolase and Thr dehydrogenase, thereby conserving the energy used in the synthesis of endogenous Gly, thus, leading to increased amount of available energy for optimal performance (Corzo et al. 2009; Lee et al. 2014).
Dietary SID Thr levels did not affect BWG ($P = 0.49$) but influenced feed intake ($P = 0.04$) and feed:gain ($P = 0.001$) as shown in Table 2. Lower feed intake and feed:gain were obtained in birds fed 8.1 g/kg SID Thr diet when compared to those fed diets containing 6.9 and 9.3 g/kg SID Thr. The current study proved that requirement of SID Thr at 8.1 g/kg in low CP diet is sufficient to support performance in broilers at their early growth phase. This is close accordance with findings of Valizade et al. (2016) and Chen et al. (2017) who reported a requirement of 8.0 g/kg SID Thr in broilers chicken at 21 d. The decreased feed:gain obtained in birds fed adequate SID Thr may be linked to the fact that Thr increased nutrient digestion owing to greater amylase secretion in the digestive tract as well as its role to stabilising and maintaining intestinal tract, which is significantly associated to mucins production, amino acid backbone (Schaart et al. 2005; Najafi et al. 2017). Increasing dietary levels of Glyequi resulted in a quadratic effect for BWG ($P = 0.02; R^2 = 0.96$) and feed:gain ($P = 0.06; R^2 = 0.98$) with an estimated optimum point of 17.2 and 16.7 g/kg Glyequi, respectively (Table 2). The result supports the claims of previous researchers (Corzo et al. 2005; Dean et al. 2006; Ospina-Rojas et al. 2012), who reported that the requirement for Glyequi in low CP diet formulated exclusively on vegetable ingredients for 21 d old broilers is far higher than the recommended level of 1.25% proposed by NRC (1994). Earlier reports have demonstrated that adequate Glyequi concentration positively affect low CP diets by improving efficiency of essential AA utilisation especially in practical corn-soybean based diet (Berres et al. 2010; Yuan et al. 2012; Ospina-Rojas et al. 2013b).

An interaction between Glyequi and SID Thr levels was not observed for all serum metabolites evaluated in the present study except for SUA (Table 4). The SUA showed a positive linear trend in response to increasing Glyequi levels in diet containing 6.9 g/kg SID Thr concentration ($P = 0.001$, $R^2 = 0.97$). However, there were no linear or quadratic effects of SUA in response to increasing Glyequi levels for diets containing 8.1 and 9.3 g/kg SID Thr concentrations. When the interaction observed between SID Thr diets and Glyequi levels were expanded (Table 5), birds offered diets with 6.9 g/kg SID Thr concentration had lower ($P < 0.05$) SUA at 18.8 g/kg Glyequi than those fed 12.8 g/kg Glyequi. When the SID Thr-adequate diet (8.1 g/kg) containing 15.8 and 12.8 g/kg Glyequi were fed, birds in these group recorded lower ($P < 0.05$) SUA concentration compared to those on 16.9, 17.3 and 18.8 g/kg Glyequi. Moreover, in diet containing excess SID Thr concentration (9.3 g/kg), lower ($P < 0.05$) SUA concentration was observed at 18.8, 15.8 and 12.8 g/kg Glyequi than those offered 16.9 g/kg Glyequi. Nevertheless, a lower ($P < 0.05$) SUA concentration was observed in birds when offered 12.8, 14.3 and 15.8 g/kg Glyequi diet containing adequate (8.1 g/kg) Glyequi/8.1 g/kg Thr Glyequi/9.3 g/kg Thr Glyequi x Thr 0.62 0.84 0.68 0.01 0.78 0.34

### Table 4. Serum biochemical metabolites of broilers on low protein diet (LPD) containing different dietary Glycine equivalent (Glyequi) level with varying concentrations of Standardized Ileal Digestible threonine (SID Thr) at 21 d of age.

| Glyequi (g/kg) | GLU | TP | ALB | SUA | CRE | TRG |
|---------------|-----|----|-----|-----|-----|-----|
| 12.8          | 6.9 | 243.09 | 2.73 | 1.60 | 4.90 | 0.22 | 82.31 |
| 14.3          | 6.9 | 244.32 | 2.66 | 1.50 | 4.39 | 0.22 | 88.21 |
| 15.8          | 6.9 | 241.77 | 2.58 | 1.47 | 4.13 | 0.25 | 79.45 |
| 17.3          | 6.9 | 255.88 | 2.90 | 1.61 | 3.95 | 0.21 | 87.87 |
| 18.8          | 6.9 | 267.82 | 2.79 | 1.67 | 3.43 | 0.23 | 83.77 |
| 12.8          | 8.1 | 243.65 | 2.74 | 1.55 | 3.19 | 0.24 | 83.97 |
| 14.3          | 8.1 | 267.25 | 2.90 | 1.70 | 3.83 | 0.23 | 89.01 |
| 15.8          | 8.1 | 287.23 | 2.74 | 1.63 | 3.00 | 0.26 | 79.94 |
| 17.3          | 8.1 | 280.53 | 2.90 | 1.62 | 4.70 | 0.23 | 88.52 |
| 18.8          | 8.1 | 256.68 | 2.93 | 1.55 | 3.82 | 0.23 | 83.50 |
| 12.8          | 9.3 | 259.17 | 2.67 | 1.54 | 3.91 | 0.27 | 81.56 |
| 14.3          | 9.3 | 266.60 | 2.75 | 1.56 | 4.58 | 0.23 | 85.61 |
| 15.8          | 9.3 | 284.83 | 3.11 | 1.66 | 3.79 | 0.25 | 80.30 |
| 17.3          | 9.3 | 262.47 | 2.44 | 1.76 | 4.03 | 0.26 | 78.07 |
| 18.8          | 9.3 | 260.66 | 2.72 | 1.57 | 3.73 | 0.25 | 73.47 |
| SEM           |     | 14.60 | 0.27 | 0.09 | 0.22 | 0.02 | 4.70 |

### Table 5. Interaction effects of Glycine equivalent (Glyequi) and Standardized Ileal Digestible threonine (SID Thr) levels on uric acid (1–21 d).

| Glyequi (g/kg) | SID Thr (g/kg) |
|---------------|----------------|
| Glyequi/6.9 g/kg Thr | – | – | – | $L = 0.001$ | – | – |
| Glyequi/8.1 g/kg Thr | – | – | – | 0.59 | – | – |
| Glyequi/9.3 g/kg Thr | – | – | – | 0.88 | – | – |

*Means in columns followed by different uppercase superscripts are statistically different ($P < 0.05$). L = Linear effect; Q = Quadratic effect.

$y = 7.7203 - 0.2253Glyequi$; $R^2 = 0.97$.

### Table 5. Interaction effects of Glycine equivalent (Glyequi) and Standardized Ileal Digestible threonine (SID Thr) levels on uric acid (1–21 d).

| Glyequi (g/kg) | SID Thr (g/kg) |
|---------------|----------------|
| Glyequi/6.9 g/kg Thr | 12.8 | 14.3 | 15.8 | 17.3 | 18.8 |
| Glyequi/8.1 g/kg Thr | 6.9 | 4.90 | 4.39 | 4.13 | 3.95 | 3.43 |
| Glyequi/9.3 g/kg Thr | 8.1 | 3.19 | 3.83 | 3.05 | 4.70 | 3.82 |
| Glyequi/9.3 g/kg Thr | 9.3 | 3.91 | 4.58 | 3.79 | 4.03 | 3.73 |

*Means in rows followed by different lowercase superscripts are statistically different ($P < 0.05$).

*Means in columns followed by different uppercase superscripts are statistically different ($P < 0.05$).
kg) SID Thr level compared to those fed the same diets containing deficient (6.9 g/kg) or excess (9.3 g/kg) SID Thr levels. However, birds fed diet containing 17.3 g/kg Glyequi showed a lower ($P < 0.05$) SUA concentration when SID Thr was in deficient (6.9 g/kg) or excess (9.3 g/kg) of its requirement. At higher dietary Glyequi concentration of 18.8 g/kg, there was no difference ($P < 0.05$) observed in SUA of the birds with increasing dietary SID Thr levels. According to Donsbough et al. (2010), serum concentration of uric acid could be used as influential indicator to reflect nitrogen utilisation in birds. The linear reduction in SUA with increasing levels of Glyequi without any evidence of plateau in diet containing lower SID Thr concentration showed that the requirement of dietary Glyequi not lower than 18.8 g/kg is necessary to enhance the efficiency of AA utilisation. This is in accordance with the report of Ospina-Rojas et al. (2012) and Wang et al. (2020), who observed that sufficient Gly supplementation has the potential to spare several dispensable AA and consequently enhanced nitrogen utilisation in low CP diets. Gly is very important in the formation of uric acid molecules to eliminate nitrogen excess (Ngo et al. 1977; Corzo et al. 2005). In addition, the decrease in SUA in birds fed low SID Thr diet in response to increased Glyequi levels is an indication of decreased rate of AA oxidation in order to spare more protein for efficient utilisation (Akinde 2014). This could be attributed to the fact that uric acid is the final product of protein catabolism in chicken and several studies have shown that reduction of dietary CP level with adequate essential and non-essential AAs resulted to lower uric acid concentration (Swennen et al. 2006; Namroud et al. 2008; Hernandez et al. 2012).

There was no interaction ($p < 0.05$) between levels of Glyequi and SID Thr on pectoral muscle creatine (PMC) and relative weights of the breast meat, liver and pancreas of the birds (Table 6). The main effect of SID Thr levels did not show any effect ($P > 0.05$) on the

| Glyequi, g/kg | SID Thr g/kg | Muscle creatine mg/g | Breast (g/kg BW) | Liver (% BW) | Pancreas (% BW) |
|-------------|-------------|----------------------|------------------|--------------|-----------------|
| Glyequi, g/kg | SID Thr g/kg | Muscle creatine mg/g | Breast (g/kg BW) | Liver (% BW) | Pancreas (% BW) |
| 12.8 | 6.9 | 2.93 | 128.22 | 3.39 | 0.334 |
| 14.3 | 6.9 | 3.49 | 136.33 | 3.49 | 0.328 |
| 15.8 | 6.9 | 3.83 | 121.03 | 3.38 | 0.344 |
| 17.3 | 6.9 | 3.40 | 128.37 | 3.81 | 0.324 |
| 18.8 | 6.9 | 3.48 | 133.20 | 3.67 | 0.341 |
| 12.8 | 8.1 | 2.99 | 125.82 | 3.61 | 0.411 |
| 14.3 | 8.1 | 3.53 | 124.46 | 3.30 | 0.371 |
| 15.8 | 8.1 | 3.60 | 131.18 | 3.22 | 0.338 |
| 17.3 | 8.1 | 3.88 | 142.93 | 2.57 | 0.393 |
| 18.8 | 8.1 | 3.98 | 133.69 | 3.66 | 0.294 |
| 12.8 | 9.3 | 3.17 | 127.22 | 3.51 | 0.309 |
| 14.3 | 9.3 | 3.43 | 127.54 | 3.10 | 0.335 |
| 15.8 | 9.3 | 3.94 | 139.06 | 3.14 | 0.392 |
| 17.3 | 9.3 | 3.95 | 124.56 | 3.42 | 0.348 |
| 18.8 | 9.3 | 3.74 | 135.07 | 3.26 | 0.362 |
| SEM | 0.27 | 4.94 | 0.26 | 0.03 |
| Glyequi, g/kg | SID Thr, g/kg | Muscle creatine mg/g | Breast (g/kg BW) | Liver (% BW) | Pancreas (% BW) |
| 12.8 | 3.03 | 127.09 | 3.50 | 0.381 |
| 14.3 | 3.49 | 129.45 | 3.30 | 0.345 |
| 15.8 | 3.79 | 130.42 | 3.25 | 0.358 |
| 17.3 | 3.74 | 131.95 | 3.27 | 0.355 |
| 18.8 | 3.73 | 133.99 | 3.33 | 0.332 |
| SEM | 0.16 | 2.85 | 0.15 | 0.02 |

Anova

| Glyequi/6.9 g/kg SID Thr | Glyequi/8.1 g/kg SID Thr | Glyequi/9.3 g/kg SID Thr |
|--------------------------|--------------------------|--------------------------|
| ns | ns | ns |

$L$ = Linear effect; $Q$ = Quadratic effect.

$y = -8.221 + 1.404Gly_{equi} - 0.041Gly_{equi}^2; R^2 = 0.7378$; Optimal $Gly_{equi} = 17.1 g/kg$. 
$y = 113.41 - 1.0867Gly_{equi}; R^2 = 0.88$. 

Table 6. Pectoral muscle creatine and carcase traits of broilers on low protein diet containing different dietary Glycine equivalent (Glyequi) level with varying concentrations of Standardized Ileal Digestible threonine (SID Thr).
PMC and breast meat weight as well as liver weight. This is in agreement with the report of earlier studies (Dozier et al. 2000; Azzam and El-Gogary 2015; Jiang et al. 2018) showing that dietary Thr levels did not show any significant differences (p > 0.05) in their effects on the carcase yield and weights of liver, gizzard, and pancreas. Increasing dietary Gly_{equi} levels resulted in a quadratic effect (p ≤ 0.03) on PMC with an optimal level estimated at 17.1 g/kg Gly_{equi} (Table 6). In the present study, the result shows that optimum dietary Gly_{equi} level at 17.1 g/kg is necessary to improve the concentration of muscle creatine that would enhanced the capacity of birds to maintain energy homeostasis and increase muscle growth. Creatine is a constituent of central importance in the energy metabolism particularly of muscle cells and assists in maintaining energy homeostasis in muscles (Lemme et al. 2007; Guimarães-Ferreira 2014). Diets formulated exclusively on plant based feedstuffs supply less creatine than required by poultry, thereby increasing the requirement of Gly for creatine synthesis. Consequently, adequate Gly supplementation in broiler diets would result to a greater capacity of the skeletal muscle to store creatine needed to improve energy utilisation for muscle growth (Michiels et al. 2012; Ospina-Rojas et al. 2013a; DeGroot et al. 2018; Majdeddin et al. 2020). On the other hand, the lower concentration of creatine in the pectoral muscle of birds fed 12.8 g/kg Gly_{equi} might be an indication that deficiency of Gly_{equi} could limit creatine formation in broiler chickens fed vegetable based low-CP supplemented AA diets. A positive linear response (p < 0.05) of dietary Gly_{equi} levels on relative breast weight of the broilers was recorded, showing that an increase in Gly_{equi} levels up to 20.6 g/kg resulted to a corresponding increase in the relative weight of breast meat. Our result is in agreement with the findings of Ospina-Rojas et al. (2013a) who also observed similar increase in relative breast weight of broilers fed increased level of dietary Gly, reporting that the increase in breast meat weight might be a result of the increase in the concentration of the muscle creatine in response to supplemental Gly, because creatine is essential to support energy metabolism.

**Conclusion**

The minimum requirement of Gly_{equi} in low-CP diet with excess SID Thr concentrations is 15.5 g/kg; but in diets containing low or adequate SID Thr concentration, a minimum of 18.8 g/kg Gly_{equi} is required for broiler chickens during starter period (1–21 days of age) for enhanced feed efficiency and growth.

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**Ethical approval**

This study was approved by the Animal Care and Ethics Committee of the Universidade Estadual de Maringá, Brazil for the use of animals in research (Protocol no: CEUA 7501230419).

**Disclosure statement**

The authors declare that there are no conflicts of interest.

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**ORCID**

Paschal C. Aguihe http://orcid.org/0000-0003-3639-9079
Kazuo A. Hirata http://orcid.org/0000-0003-2906-035X
Camilo I. Ospina-Rojas http://orcid.org/0000-0001-6457-3535
Tatiana C. dos Santos http://orcid.org/0000-0003-1463-7785
Paulo C. Pozza http://orcid.org/0000-0001-8453-7371
Eustace A. Iyayi http://orcid.org/0000-0001-5097-8072
Alice E. Murakami http://orcid.org/0000-0002-8872-4550

**Data availability statement**

The result and analyses presented in this paper freely available upon request.

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