Supplementing glycine, serine, and threonine in low protein diets for meat type chickens

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ABSTRACT Reducing dietary protein has been of interest to the global poultry industry to improve bird health, welfare, and industry sustainability. Low protein (**LP**) diets are typically glycine (**Gly**) deficient and produce poor performance. Supplementing the diet with Gly or precursors of Gly can overcome this deficiency. A feeding experiment was conducted with 330 Ross 308 off-sex males across 5 treatments in a randomized design using 11 birds per pen replicated 6 times. Grower and finisher treatments were fed from day 7 to 21 and day 21 to 35, respectively. The objective was to test the efficacy of supplementation with Gly and Gly equivalents (**Gly$_{equiv}$**), serine (**Ser**) and threonine (**Thr**), in plant-based LP diets on bird performance against a standard protein (**SP**) diet containing meat and bone meal. Glycine, Ser, or Thr were supplemented on Gly$_{equiv}$ basis to an approximately 3% lower CP diet to achieve the same digestible Gly and Ser level as the SP diet. Nitrogen efficiency, serum uric acid, blood plasma amino acids (AA) and AA digestibility were also investigated to monitor potential metabolic effects. Birds fed the LP diet were only 3.3% lower in final body weight than the SP treatment (2,556 vs. 2,641 g) while the supplementation of Gly or Ser had no effect. Supplementation of Thr reduced final body weight by 9.5% ($P < 0.05$). Reducing CP increased N efficiency by 9.6% ($P < 0.05$) and decreased blood serum uric acid by 26.9% ($P < 0.001$) in the finisher treatments. Glycine and Ser supplementation in LP diets had no effect on these parameters. The LP diet reduced AA digestibility and blood plasma AA while the supplementation with either Gly, Ser, or Thr increased overall AA digestibility ($P < 0.05$) but had no overall effect on blood plasma AA. Further research is required into Gly metabolism; however, Thr supplementation depressed growth and therefore is not feasible to cover Gly deficiency in LP diets on a Gly$_{equiv}$ basis.

Key words: Key words meat chickens, low protein, glycine, glycine equivalents

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INTRODUCTION

Low protein (**LP**) diets have been identified to potentially reduce feed costs, environmental impacts, health, and welfare concerns, and increase feed utilization (Hilliar and Swick, 2018). Low protein diets supplemented with essential amino acids (**AA**) have failed to maintain performance objectives observed in standard diets. However, supplementation of the nonessential AA glycine (**Gly**) in LP diets can improve performance (Corzo et al., 2005; Dean et al., 2006; Ospina-Rojas et al., 2013a). Diets low in CP have been observed to decrease blood uric acid and increase blood ammonia compared to high CP diets (Namroud et al., 2008). A diet limiting in Gly is believed to trigger this as Gly is required for the metabolization of ammonia into uric acid. A high level of blood ammonia has been observed to suppress appetite (Panksepp and Booth, 1971) and is the mechanism believed to be behind Gly deficiency and impaired performance in LP diets (Namroud et al., 2008). Glycine is also involved in the synthesis of collagen, heme, glutathione, and creatine (Kidd and Kerr, 1996; Stevens, 1996; Shoulders and Raines, 2009). Insufficient Gly impairs growth, supporting the term of conditionally essential in birds due to the key roles it plays in growth and function (Wang et al., 2013).

Glycine is known to be an in vivo product of both threonine (**Thr**) and serine (**Ser**) metabolism, suggesting supplementation of these AA may help satisfy Gly requirements (Dean et al., 2006; Ospina-Rojas et al., 2013b). Threonine is degraded to Gly by Thr dehydrogenase and Thr aldolase (Baker and Sugahara, 1970). Ospina-Rojas et al. (2013a), observed increased performance when increasing dietary Thr in LP diets suggesting the *in vivo* reactions are taking place in birds fed LP

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diets deficient in Gly. Serine is a nonessential AA that is interconverted to Gly in the presence of Ser hydroxymethyltransferase, therefore both AA are considered as Gly equivalents (**Gly_{equiv}**) in diet formulations. The complete supplementation of Thr or Ser to meet inadequate dietary Gly_{equiv} levels has not been investigated. If this approach is achievable in meeting Gly_{equiv}, diet formulations that consider Thr and Ser levels as Gly_{equiv} can contribute to the successful extension of LP diets in industry. Additionally, meat and bone meal (**MBM**) is high in Gly (Kim et al., 2012) and when used in commercial broiler diets it typically satisfies the Gly_{equiv} requirements of growing meat chickens. The use of crystalline AA offers a replacement of MBM, removing the reliable source of dietary Gly from broiler diets. The removal of MBM from broiler diets is also favored due to reduced AA digestibility as a result of MBM procession, contributing to diet formulation errors (Batterham et al., 1986). This can also increase the risk of necrotic enteritis (Drew et al., 2004) as more undigested protein enters the hindgut. Furthermore, the use of MBM is also restricted in Europe as a result of Bovine spongiform encephalopathy outbreaks (Ducrot et al., 2013). Therefore, the current experiment was conducted to assess the implications of supplementing Gly_{equiv} requirements of LP diets with the Gly precursors Thr and Ser to the level of Gly_{equiv} offered in a commercial SP diet including MBM.

MATERIALS AND METHODS

Experimental Design and Diets

A total of 330 Ross 308 males from the female breeder line (off-sex) from Aviagen hatchery in Goulburn, Australia were brought to the Centre of Animal Research and Teaching at the University of New England, Armidale, Australia. All experimental procedures were approved by the University of New England Animal Ethics Committee (AEC17–042). On day 7, birds were assigned to 30 equal sized floor pens (120 × 75 cm) across 5 treatments based on overall average bird weight with 5% variation and checked for no significant differences between treatments ($P > 0.05$). Each treatment was replicated 6 times and 11 chicks were allocated to each pen with wood shavings as bedding material. The birds were raised in temperature-controlled rooms. All birds were fed a common starter diet (3000 kcal/kg, 25% CP) containing wheat, sorghum, soybean meal, and MBM from day 0 to 7. As per Australian practice, zinc bacitracin was provided in the starter diet at 0.05%. The dietary treatments investigated two protein levels as shown in Table 1 that included a standard protein (**SP**) diet containing MBM and a LP vegetable-based diet that was 3 percentage points lower in CP. For the purpose of this experiment a SP diet is defined as a diet with a CP level that only requires three essential AA supplemented to meet nutrient specifications. Additionally, the LP diet was supplemented with either Gly, Ser or Thr to 1.6% dietary Gly_{equiv} in replacement of wheat, as shown in Table 2. This resulted in a total of 5 dietary treatments. The Gly_{equiv} level was formulated to equal the amount in the SP treatment, with Gly, Ser, and Thr supplemented on an equimolar basis. Serine was converted to a unit of Gly_{equiv} at 0.7143 × Ser (%) following Dean et al. (2006); Thr was equated to a Gly_{equiv} by 0.6302 × Thr (%) following the same molar conversion principle. This level of Gly_{equiv} was chosen to match that formulated to be in the SP treatment with MBM. The control treatment was formulated to represent an industry standard diet supplemented only with the AA; D, L-methionine, L-lysine HCl, and L-Thr. The LP diets were further supplemented with L-valine, L-arginine, and L-isoleucine to meet the AA specifications. AMINOChick 2.0 software (Evonik Nutrition and Care GmbH, 2017) was used to calculate AA specifications of the diets with N-corrected metabolic energy set at 3100 kcal/kg for grower (day 7 to 21) treatments and 3200 kcal/kg for finisher (day 21 to 35) treatments. The diets were formulated to be isoenergetic across all treatments. Additionally, to ensure excess choline was not metabolized to Gly (Siegert et al., 2015), choline chloride was not added to SP treatment diets, and only supplemented in LP diets to the formulated level in SP treatment diets.

Wheat, sorghum, soybean meal, and canola oil were used as ingredients in the diet formulations, with MBM used in the SP diet formulation only. Grower treatments were fed from day 7 to 21 and finisher treatments from day 21 to 35. The nutrient content of ingredients was estimated using near-infrared spectroscopy (Foss NIR 6500, Denmark) standardized with Evonik AMINONIR Advanced calibration. Xylanase (Econase XT 25, AB Vista) and phytase (Quantum Blue, 5 G, AB Vista) enzymes were added to the diets at 1000 BXU/kg and 500 FTU/kg, respectively. Vitamin and mineral premixes were added to meet requirements following manufacturer recommendations (UNE VM, Rabar Pty Ltd; UNE TM, Rabar Pty Ltd). Amino acid and energy matrix values for phytase (500 FTU/kg) were considered in diet formulations as recommended by the manufacturer. Titanium dioxide was formulated into the diets as an inert marker for digestibility assay at 0.5%.

Data Measurement

Chickens and feed were weighed weekly from day 7 to 35 to measure feed intake and pen body weight. Mortalities were recorded and the feed conversion ratio (**FCR**) was calculated with correction for mortalities by adding the weight of dead birds to live birds for each period. Birds were randomly sampled on day 21 and 35 at 3 and 2 birds per pen respectively and were euthanized by electrical stunning and decapitation for sample collection. On day 21, blood and ileum contents were collected for uric acid and AA digestibility assays, respectively. On day 35, blood was collected for uric acid and AA analysis and breast meat yield was measured by removing the left breast from each chicken. The ileum was defined as the small intestine between the Meckel's

Table 1. Diet formulations, nutrient content, and analyzed values.

Ingredients, %	Grower control	Grower low protein (LP)	Finisher control	Finisher LP
Wheat (10.6% CP)	42.74	52.08	46.05	56.06
Soybean meal (45.7% CP)	27.39	20.99	24.90	16.80
Sorghum (11.7% CP)	20.00	20.00	20.00	20.00
Meat and bone meal (52.7% CP)	4.62	0.00	2.72	0.00
Canola oil	3.06	2.33	4.14	3.11
Dicalcium phosphate	0.00	0.96	0.00	0.59
Limestone	0.71	1.23	0.73	1.01
Salt	0.08	0.14	0.10	0.13
Sodium bicarbonate	0.15	0.15	0.15	0.15
Xylanase[1]	0.005	0.005	0.005	0.005
Phytase[2]	0.010	0.010	0.010	0.010
Titanium dioxide	0.500	0.500	0.500	0.500
Vitamin/mineral UNE premix[3]	0.180	0.180	0.160	0.160
L-lysine HCl	0.205	0.478	0.201	0.482
D,L-methionine	0.255	0.303	0.225	0.275
L-threonine	0.072	0.181	0.066	0.179
L-isoleucine	0.000	0.119	0.000	0.131
L-arginine	0.000	0.146	0.000	0.176
L-valine	0.000	0.148	0.000	0.151
Choline chloride	0.000	0.031	0.000	0.031
Salinomycin (11.7%)	0.050	0.050	0.050	0.050
Nutrient composition, %				
MEn, kcal/kg	3100	3100	3200	3200
CP[4]	22.7 (22.3)	19.1 (18.6)	20.9 (19.9)	17.7 (17.2)
Dig[5] Lys	1.110	1.110	1.020	1.020
Dig Met	0.520	0.529	0.475	0.487
Dig TSAA	0.820	0.820	0.770	0.770
Dig Thr	0.710	0.710	0.660	0.660
Dig Trp	0.245	0.213	0.232	0.195
Dig Val	0.880	0.880	0.820	0.820
Dig Ile	0.775	0.770	0.725	0.720
Dig Leu	1.414	1.190	1.322	1.090
Dig Phe	0.910	0.767	0.852	0.699
Dig Arg	1.291	1.150	1.175	1.070
Dig His	0.466	0.391	0.435	0.356
Dig Gly	0.928	0.601	0.793	0.550
Dig Ser	0.889	0.758	0.835	0.695
Dig Gly_{equiv}	1.563	1.142	1.390	1.046
Lys	1.259 (1.285)	1.204 (1.141)	1.146 (1.135)	1.103 (1.063)
Met	0.556 (0.579)	0.553 (0.492)	0.505 (0.473)	0.509 (0.445)
Cys	0.360 (0.326)	0.335 (0.291)	0.363 (0.305)	0.331 (0.276)
TSAA	0.916 (0.905)	0.888 (0.783)	0.855 (0.778)	0.832 (0.721)
Thr	0.843 (0.862)	0.802 (0.741)	0.776 (0.943)	0.741 (0.722)
Trp	0.281 (0.261)	0.240 (0.222)	0.264 (0.236)	0.220 (0.207)
Val	1.018 (0.985)	0.974 (0.898)	0.940 (0.891)	0.904 (0.841)
Ile	0.884 (0.871)	0.845 (0.782)	0.820 (0.785)	0.785 (0.728)
Leu	1.623 (1.604)	1.339 (1.311)	1.507 (1.425)	1.224 (1.207)
Phe	1.033 (1.041)	0.856 (0.847)	0.961 (0.919)	0.778 (0.791)
Arg	1.446 (1.408)	1.255 (1.170)	1.310 (1.218)	1.168 (1.086)
His	0.529 (0.490)	0.438 (0.416)	0.492 (0.455)	0.398 (0.383)
Gly	1.223 (1.184)	0.770 (0.764)	1.037 (0.908)	0.709 (0.667)
Ser	1.037 (1.001)	0.855 (0.812)	0.962 (0.901)	0.781 (0.749)
Gly_{equiv}	1.964 (1.899)	1.402 (1.344)	1.724 (1.552)	1.267 (1.202)
Calcium	0.900	0.920	0.750	0.750
Available phosphorus	0.454	0.454	0.380	0.380
Choline, mg/kg	1431	1432	1362	1362
Sodium	0.167	0.164	0.161	0.160
Chloride	0.160	0.230	0.160	0.227
DEB[6], mEq/kg	258	206	243	186
Linoleic acid	1.694	1.519	1.966	1.713

[1]Econase XT, 25, AB Vista.
[2]Quantum Blue, 5 G, AB Vista.
[3]Vitamin premix per kg diet: vitamin A, 12 MIU; vitamin D, 5 MIU; vitamin E, 75 mg; vitamin K, 3 mg; nicotinic acid, 55 mg; pantothenic acid, 13 mg; folic acid, 2 mg; riboflavin, 8 mg; cyanocobalamin, 0.016 mg; biotin, 0.25 mg; pyridoxine, 5 mg; thiamine, 3 mg; antioxidant, 50 mg. Mineral premix per kg diet: Cu, 16 mg as copper sulfate; Mn, 60 mg as manganese sulfate; Mn, 60 mg as manganous oxide; I, 0.125 mg as potassium iodide; Se, 0.3 mg; Fe, 40 mg, as iron sulfate; Zn, 50 mg as zinc oxide; and Zn, 50 mg as zinc sulfate.
[4]Measured as-is values in parentheses.
[5]Digestible coefficients for raw ingredients determined using AMINODat 5.0 (Evonik Animal Nutrition).
[6]DEB mEq/kg calculated as 10,000 × $(Na^+ + K^+ - Cl^-)$.

Table 2. Supplementation of glycine (Gly), serine (Ser), and threonine (Thr) to basal low protein (LP) treatment diets and nutrient analysis.

Ingredients%	Grower LP Gly	Grower LP Ser	Grower LP Thr	Finisher LP Gly	Finisher LP Ser	Finisher LP Thr
Wheat, (10.6% CP)	51.7	51.5	51.4	55.7	55.6	55.5
L-Threonine	0.181	0.181	0.841	0.179	0.179	0.724
Glycine	0.416	0.000	0.000	0.343	0.000	0.000
L-Serine	0.000	0.582	0.000	0.000	0.480	0.000
Nutrient composition %						
CP[1]	19.6 (18.8)	19.6 (18.8)	19.6 (19.0)	18.1 (17.5)	18.1 (17.5)	18.1 (17.5)
Dig[2] Thr	0.710	0.710	1.369	0.660	0.660	1.204
Dig Gly	1.020	0.601	0.601	0.893	0.550	0.550
Dig Ser	0.760	1.340	0.760	0.695	1.175	0.695
Dig Gly_{equiv}	1.563	1.558	1.558	1.390	1.389	1.389
Thr	0.802 (0.766)	0.802 (0.757)	1.369 (1.220)	0.741 (0.708)	0.741 (0.697)	1.204 (1.111)
Gly	1.020 (1.025)	0.770 (0.772)	0.770 (0.707)	0.893 (0.911)	0.709 (0.683)	0.709 (0.645)
Ser	0.855 (0.805)	1.304 (1.230)	0.855 (0.905)	0.781 (0.749)	1.175 (1.092)	0.781 (0.769)
Gly_{equiv}	1.631 (1.600)	1.701 (1.651)	1.762 (1.712)	1.451 (1.446)	1.548 (1.463)	1.579 (1.563)

[1]Measured as-is values in parentheses.
[2]Digestible coefficients for raw ingredients determined using AMINODat 5.0 (Evonik Animal Nutrition).

diverticulum and 1 cm before the ileocecal junction. To avoid contaminating the digesta with intestinal secretions, the ileum was cut to collect the contents.

Feed and Digesta Amino Acid and Titanium Dioxide Analysis

Digesta samples were stored at −20°C and prepared for analysis by freeze-drying (Christ Alpha 1–4 LDplus, Osterode am Harz, Germany) and grinding. Ground feed and digesta samples were analyzed for CP by Dumas method and AA profile at AMINOLab, Singapore (Evonik SEA). The AA analysis was done by standard procedures (method 994.12 Association of Official Analytical Chemists, 1994) of oxidation followed by hydrolysis and the AA were detected using an AA analyzer (Biochrom 30+, Cambridge, UK). Diet analysis for AA profile and CP are in good agreement with formulated values as shown in Table 1 and Table 2. Titanium was measured in duplicate for diets and digesta samples by calorimetric method (Short et al., 1996).

Serum Uric Acid Analysis

Blood serum was collected for serum uric acid (**SUA**) analysis at day 21 and 35 in Vacutainers that contained spray-coated silica and a polymer gel for serum separation and stored at 4°C until centrifuged. The samples were centrifuged at 1500 × g at 4°C for 5 min to separate the serum and stored at −20°C until analysis. The samples were then analyzed for uric acid concentration using an integrated chemistry analyzer (Siemens Dimension Xpand Plus, Newark, NJ, US).

Plasma Amino Acid Analysis

Blood plasma was collected at day 35 using Vacutainers spray-coated with sodium heparin and stored at 4°C until centrifuged. The samples were centrifuged at 1500 × g at 4°C for 10 min for plasma separation and stored at −20°C. Samples were then freeze-dried at the University of New England for transport to meet customs regulations. The samples were reconstituted using distilled water to the original plasma volume before analysis. Blood plasma AA were measured by an Ion exchange chromatography AA analyzer (Biochrom 30+, Cambridge, UK) using norleucine as an internal standard at Evonik Nutrition and Care GmbH laboratory, Hanau–Wolfgang, Germany.

Calculations and Statistics

Amino acid and CP digestibility were calculated using the equation as described by Gracia et al. (2007):

$$X_{dig}(\%) = 100 - \left(\frac{Ti_{Diet} \times X_{Digesta}}{Ti_{Digesta} \times X_{Diet}}\right) \times 100$$

Calculation of FCR for each pen was achieved by using:

$$\text{FCR (g/g)} = \frac{\text{Total pen feed intake}}{(\text{Final pen weight} - \text{initial pen weight}) + \text{pen mortality weight}}$$

To calculate feed intake per bird:

$$\text{Feed intake (g/bird)} = \text{FCR (g/g)} \times \text{average body weight gain (g/bird)}$$

Nitrogen efficiency was calculated for each pen using equations described by Belloir et al. (2017) by dividing nitrogen (**N**) retention by N intake multiplied by 100. Nitrogen intake was calculated by multiplying feed intake per bird by CP of the diet as a percentage of 1 divided by 6.25. Nitrogen retention was calculated using a constant for whole body N of 29 g/kg (ITAVI,

Table 3. Performance results including feed intake, body weight gain, feed conversion ratio (FCR), livability, relative breast weight, and nitrogen efficiency for grower (day 7 to 21), finisher (day 21 to 35), and overall (day 7 to 35).

Treatment	Standard protein	Low Protien (LP)	LP glycine	LP serine	LP threonine	SD	SEM	*P*-Value
Grower feed intake (g)	1090[b]	1170[a]	1115[a,b]	1127[a,b]	1066[b]	52	9	0.002
Finisher feed intake (g)	2300	2330	2320	2229	2175	148	27	0.319
Overall feed intake (g)	3382	3462	3397	3320	3201	175	32	0.097
Body weight at day 7 (g)	170	170	167	170	167	3	1	0.186
Grower body weight gain (g)	879[a]	866[a]	836[a,b]	844[a,b]	801[b]	38	7	0.001
Finisher body weight gain (g)	1595	1515	1520	1502	1421	105	20	0.066
Overall body weight gain (g)	2471[a]	2385[a,b]	2362[a,b]	2354[a,b]	2223[b]	136	25	0.022
Body weight at day 35 (g)	2641[a]	2556[a,b]	2529[a,b]	2524[a,b]	2391[b]	137	25	0.022
Grower FCR (g/g)	1.272[a]	1.351[b]	1.335[b]	1.352[b]	1.339[b]	0.036	0.007	<0.001
Finisher FCR (g/g)	1.444[a]	1.535[b]	1.520[b]	1.482[a,b]	1.530[b]	0.049	0.009	0.001
Overall FCR (g/g)	1.354[a]	1.452[b]	1.438[b]	1.417[b]	1.440[b]	0.041	0.008	<0.001
Overall livability[1] (%)	92	97	97	94	94	6	1	0.672
Breast (%)	11.37[a]	10.59[a,b]	10.03[b]	9.92[b]	9.97[b]	0.72	0.13	<0.001
Day 7 to 21 N_{eff}[2] (%)	64.42[b]	70.27[a]	69.28[a]	69.30[a]	69.49[a]	2.48	0.45	<0.001
Day 21 to 35 N_{eff}[2] (%)	60.21[b]	66.61[a]	65.64[a]	65.17[a]	65.43[a]	3.44	0.63	0.004

[1]Non-normal data were log transformed and analyzed for significance with Kruskal–Wallis non-parametric test.
[2]Nitrogen efficiency.
[a,b]Differing superscripts indicate significant differences between means determined by one-way ANOVA with Tukey post-hoc test.

2013) multiplied by bird weight gain divided by 1,000.

$$N_{intake}\,(g/bird) = \text{Feed intake}\ (g/bird) \times \frac{CP_{diet}\,(\%)}{6.25}$$

$$N_{body} = 29\ (g/kg)$$

$$N_{ret}\,(g/bird) = N_{body}\,(g/kg) \times \left(\frac{\text{Body weight gain}\ (g/bird)}{1000}\right)$$

$$N_{eff}\ (\%) = 100 \times \frac{N_{ret}\ (g/bird)}{N_{intake}\ (g/bird)}$$

The IBM SPSS statistical package (v. 24.0.0.0) was used to determine statistical significance. Data were subjected to one-way ANOVA and means were separated by Tukey's test at $P < 0.05$ when significant. Non-normal livability data were analyzed for significance with Kruskal−Wallis non-parametric test.

RESULTS AND DISCUSSION

The SP diet with MBM had the lowest FCR during both the grower and finisher phases at 1.272 ($P < 0.001$) and 1.444 g/g ($P = 0.001$), respectively. No significant difference was observed in body weight gain or final body weight between SP and LP treatments across grower and finisher phases. Feed intake was 6.8% higher in the LP treatment for grower phase compared to the SP treatment ($P < 0.01$). The difference between the SP and LP treatments suggests that nutrients contained in MBM not investigated in this experiment were limiting in the LP treatments. A difference observed in diet formulations was the dietary electrolyte balance (**DEB**) between the SP and LP treatments, with LP diet 20% lower in DEB. A lower DEB has been reported to reduce phytase activity (Ravindran et al., 2008), however this may not be the only factor to impair performance. As these diets contained phytase, a similar effect could occur in the study. The supplementation of Gly or Ser to 1.6% Gly_{equiv} did not affect feed intake, body weight gain, final body weight, or FCR in the grower treatments (day 7 to 21) in comparison to birds fed the non-supplemented LP diet as shown in Table 3. The supplementation of Thr to 1.6% Gly_{equiv} had performance-decreasing effects on grower phase feed intake ($P < 0.01$) and body weight gain ($P = 0.001$), final body weight ($P < 0.05$), and overall body weight gain ($P < 0.05$). Grower, finisher and overall feed intake, body weight gain, final body weight, and FCR showed no difference between non-supplemented LP and Gly supplemented LP treatments suggesting that the Gly_{equiv} level of 1.1 to 1.0% in the grower and finisher LP treatments respectively were not limiting and did not impair growth. Similar performance results were observed in the finisher treatments (day 21 to 35), however, the supplementation of Ser to 1.6% Gly_{equiv} in the LP diet gave a similar FCR to that seen in the SP treatment and no significant difference was seen in body weight gain between treatments. Glycine is believed to be most important for young and rapidly growing animals (Wu and Knabe, 1994; Jackson et al., 2002), which can explain the lack of effect between Gly supplementation on performance in the finisher diets. The literature suggests a minimum of 1.8% total Gly and Ser (not converted to Gly_{equiv}) in a corn-based diet at 18 to 19% CP for birds aged day 7 to 20 day (Schutte et al., 1997; Corzo et al., 2004), the results of this study suggest 1.1% Gly_{equiv} is adequate in wheat-based diets at 18 to 19% CP from day 7 to 21. This is supported by the findings of Heger and Pack (1996) which concluded Gly_{equiv} requirement can vary with

Table 4. Apparent ileal digestibility coefficients at day 21.

Treatment	Standard protein[1]	Low Protein (LP)	LP glycine (Gly)	LP serine (Ser)	LP threonine (Thr)	SD	SEM	*P*-Value
CP	0.85[a]	0.81[b]	0.84[a]	0.85[a]	0.85[a]	0.20	0.04	<0.001
Lys	0.89[a]	0.88[b]	0.90[a]	0.91[a]	0.90[a]	0.14	0.03	<0.001
Met	0.94[a]	0.93[b]	0.94[a]	0.94[a]	0.94[a,b]	0.08	0.02	0.012
Cys	0.77[a]	0.71[b]	0.75[a]	0.77[a]	0.75[a]	0.03	0.01	<0.001
TSAA[2]	0.88[a]	0.85[b]	0.87[a]	0.88[a]	0.87[a]	0.15	0.03	<0.001
Thr	0.83[b]	0.78[c]	0.82[b]	0.83[b]	0.89[a]	0.37	0.07	<0.001
Val	0.84[a]	0.82[b]	0.85[a]	0.86[a]	0.85[a]	0.19	0.04	<0.001
Ile	0.85[a]	0.83[b]	0.86[a]	0.87[a]	0.86[a]	0.19	0.04	<0.001
Arg	0.90[a]	0.88[b]	0.90[a]	0.91[a]	0.91[a]	0.14	0.03	<0.001
Phe	0.87[a]	0.83[b]	0.87[a]	0.87[a]	0.87[a]	0.21	0.04	<0.001
His	0.86[a]	0.82[b]	0.85[a]	0.86[a]	0.86[a]	0.18	0.03	<0.001
Leu	0.86[a]	0.82[b]	0.85[a]	0.86[a]	0.85[a]	0.21	0.04	<0.001
Gly	0.81[b]	0.75[c]	0.85[a]	0.82[b]	0.80[b]	0.37	0.07	<0.001
Ser	0.83[b,c]	0.78[d]	0.81[c]	0.89[a]	0.84[b]	0.28	0.07	<0.001
Pro	0.86[b]	0.84[c]	0.88[a,b]	0.88[a]	0.88[a]	0.18	0.03	<0.001
Ala	0.84[a]	0.78[b]	0.81[a]	0.82[a]	082[a]	0.27	0.05	<0.001
Glu	0.90[a,b]	0.89[b]	0.91[a]	0.91[a]	0.91[a]	0.13	0.05	0.001
Asp	0.82	0.82	0.81	0.83	0.82	0.14	0.02	0.343

[1]One replicate was removed from SP treatment due to an extreme outlier in TiO_2 measurement.
[2]Total sulfur amino acids.
[a,b,c,d]Differing superscripts indicate significant differences between means determined by one-way ANOVA with Tukey post hoc test.

dietary CP and that birds fed higher CP diets have higher Gly_{equiv} requirements. Reducing dietary CP did not significantly decrease breast percentage of total body weight at day 35. However, the supplementation of Gly, Ser, and Thr reduced breast percentage by 1.34, 1.45, and 1.40% ($P < 0.001$) respectively, compared to the SP diet. No differences in livability were observed between treatments.

All LP treatments increased N efficiency compared to the SP treatment by up to 9.08% ($P < 0.001$) and to 10.63% ($P < 0.01$) in the grower and finisher treatments respectively. The observed increase in N efficiency agrees with the findings of Belloir et al. (2017) and supports that even under limiting AA conditions meat chickens can utilize the available nutrients efficiently as well as reducing the potential environmental impact of the poultry industry.

The addition of Gly, Ser, or Thr to the LP diet increased apparent ileal digestibility of CP. Additionally, the majority of AA measured had increased apparent ileal digestibility in LP diets with Gly, Ser, or Thr supplementation, including Lys ($P < 0.001$), Met ($P < 0.05$), Cys ($P < 0.001$), total sulfur AA ($P < 0.001$), Val ($P < 0.001$), Ile (P < 0.001), Arg ($P < 0.001$), Phe ($P < 0.001$), His ($P < 0.001$), Leu ($P < 0.001$), Pro ($P < 0.001$), Ala ($P < 0.001$), and Glu ($P = 0.001$) as depicted in Table 4. The supplementation of either Gly, Ser or Thr further increased apparent ileal digestibility of Gly, Ser, or Thr in their respective treatments ($P < 0.001$) and is most likely a result of supplementing these AA in their highly digestible crystalline forms. The poorer AA digestibility in the SP treatment can be associated with the greater amount of protein-bound AA in the diet in the form of intact protein, compared to the LP supplemented treatments that contain greater levels of highly digestible crystalline AA. Feed intake is dependent on many factors including the nutrient balance of the diet, with a diet deficient or imbalanced in AA increasing feed intake (Fisher et al., 1960). Increased feed intake has been attributed to reducing ileal digestibility of starch (Svihus and Hetland, 2001), this principle has also been seen in pigs (Moter and Stein, 2004). This effect can be seen in the non-supplemented LP treatment which had the highest feed intake during the grower period and the overall poorest digestibility of AA. Additionally, the difference in ileal AA digestibility between SP and LP treatments may also be due to differences in endogenous loses as a result of different diet ingredient matrices (Adeola et al., 2016). Alternatively, the inclusion of 4.62% MBM in the grower SP treatment can also explain the differences in increased ileal AA digestibility. Slight increases in dietary Ser and Thr are known to improve gastrointestinal health and function by promoting goblet cells in the surface epithelium (Faure et al., 2006). An interesting effect occurred in Ser digestibility between LP treatments supplemented with Gly and Thr. The supplementation of Thr was able to significantly increase Ser digestibility, this can be a result of possibly improving mucin secretion (Ospina-Rojas et al., 2013b). Additionally, the activity of the phytase used in this study is theoretically limited by the reduced inclusion level of phytate from soybean meal, reducing the potential benefits of phytase on AA digestibility (Yi et al., 1996). However, as previously discussed the LP diets with Gly, Ser, or Thr supplemented had increased apparent ileal AA digestibility across all AA compared to the non-supplemented LP treatment. These results suggest that either an effect between these AA and phytase or the phytase activity was not affected by reduced soybean meal inclusion at all. In the future, a true ileal digestibility assay would be more appropriate for studies in Gly, Ser, and Thr digestibility to account for basal endogenous AA losses as these specific AA

Table 5. Blood serum uric acid at day 21 and 35 and blood plasma amino acids at day 35 (mg/dL).

Amino acid	Standard Protein (SP)	Low Protien (LP)	LP glycine (Gly)	LP serine (Ser)	LP threonine (Thr)	SD	SEM	*P*-Value
Day 21 serum uric acid (SUA)[1]	9.61[b]	7.26[a]	7.97[a,b]	7.42[a]	7.18[a]	1.47	0.27	0.013
Day 35 SUA[1]	9.46[b]	6.92[a]	7.48[a]	6.63[a]	6.28[a]	1.47	0.27	<0.001
Lys	0.26[b]	0.37[a,b]	0.51[a,b]	0.49[a,b]	0.60[a]	0.19	0.03	0.008
Met[2]	0.08	-	0.06	0.09	0.13	0.03	0.01	0.600
Thr[3]	2.83[b]	1.99[b]	2.42[b]	2.35[b]	16.46[a]	5.91	1.08	<0.001
Val	0.91[b]	0.98[b]	1.13[a,b]	1.16[a,b]	1.33[a]	0.21	0.38	0.001
Ile	0.51	0.51	0.59	0.60	0.65	0.10	0.02	0.037
Arg	1.84[a]	1.11[b]	1.31[b]	1.16[b]	1.07[b]	0.39	0.07	<0.001
Phe	0.62[a]	0.45[b]	0.49[a,b]	0.49[a,b]	0.61[a,b]	0.11	0.02	0.012
His	0.15[a]	0.08[b]	0.09[b]	0.08[b]	0.09[b]	0.03	0.01	<0.001
Leu	0.88[a]	0.65[b]	0.72[a,b]	0.68[b]	0.76[a,b]	0.13	0.02	0.007
Gly	1.85[a,b]	1.33[c]	2.11[a,b]	1.74[b,c]	2.31[a]	0.43	0.08	<0.001
Ser	1.77[b,c]	1.41[c]	1.69[b,c]	2.18[b]	2.79[a]	0.57	0.10	<0.001
Pro	4.59	4.14	4.32	4.44	4.52	0.74	0.14	0.867
Ala	4.42	5.02	4.40	4.68	5.30	0.95	0.17	0.410
Asp	2.60	1.89	2.53	2.42	2.58	0.57	0.10	0.154
Glu	1.63	1.72	1.72	1.63	1.62	0.22	0.04	0.876
Gln	5.62	7.11	7.87	7.95	7.45	1.60	0.29	0.067
Tyr	1.14	0.92	0.92	0.85	1.17	0.24	0.04	0.047

[1]Serum uric acid.
[2]Not all replicates produced concentrations of this amino acid within the range of detection.
[3]Produced some results that exceeded the detectable range, therefore, replicates that produced this result were assigned the highest possible detectable level of 40 mg/dL.
[a,b,c]Differing superscripts indicate significant differences between means determined by one-way ANOVA with Tukey post hoc test.

are common in gastrointestinal secretions and can contribute to variation between treatments.

Reducing dietary CP reduced SUA levels in both the grower and finisher periods by 24.0% ($P < 0.05$) and 26.9% ($P < 0.001$) respectively, as described in Table 5. With less excess AA in the diet, less N was excreted resulting in lower SUA levels and were able to better utilize the dietary N which is further supported by the N efficiency results. The interaction between LP diets and lowering SUA levels has been reported in the literature and has been attributed to lower Gly levels restricting uric acid production (Namroud et al., 2008; Ospina-Rojas et al., 2013a). The similarity between the LP diet supplemented with Gly and the SP diet further supports the hypothesis proposed by (Namroud et al., 2008) that low dietary Gly_{equiv} limits uric acid synthesis due to the close metabolic relationship between Gly and uric acid synthesis (Mapes and Krebs, 1978). Ospina-Rojas et al. (2013a) also observed that higher dietary Gly increases SUA levels, further supporting the findings of this study.

Blood plasma free AA concentrations varied between treatments. Significant differences were observed with reducing CP and decreasing blood Arg ($P < 0.001$), Phe ($P < 0.05$), His ($P < 0.001$), Leu ($P < 0.01$), and Gly ($P < 0.001$), that were offered at lower levels in the LP diet (Table 1). Cysteine and total sulfur AA were measured below detectable range and therefore excluded from analysis. Glutamine (Gln) is assumed to be present at lower levels in the LP diet as the CP was reduced without Gln supplementation compared to the SP diet. However, the blood plasma Gln levels tended to be higher ($P = 0.067$). Glutamine is synthesized in birds from Glu and ammonia to detoxify ammonia (Hakvoort et al., 2017), proposing that the birds fed LP diets had high ammonia levels. Ammonia levels increase in LP diets due to the higher quantities of crystalline amino acids (Namroud et al., 2008) as well as a result of decreased uric acid synthesis, which is further evident in the lower blood SUA levels in the LP treatments. No significant difference was observed in blood plasma free Met, Pro, Ala, Asp, or Glu between SP and LP treatments. This is interesting due to the lower levels of these AA offered in the diet. The supplementation of Gly, Ser, and Thr increased their respective blood plasma concentrations in LP diets. Dietary supplementation of a LP diet with Thr produced the highest blood plasma concentrations for Lys ($P < 0.01$), Val ($P = 0.001$), Gly ($P < 0.001$), and Ser ($P < 0.001$). The increasing blood plasma levels of these AA can be associated with the higher AA digestibility coefficients seen in this treatment. The significant increase in Gly and Ser blood plasma levels in the LP treatment supplemented with Thr can also be due to the degradation of Thr to Gly *in vivo* by Thr dehydrogenase and Gly c-acetyltransferase, as well as further conversion from Gly to Ser by Ser hydroxymethyltransferase. Davis and Austic (1997), found that lowering dietary CP reduced the hepatic activity of Thr dehydrogenase and therefore lower levels of Gly synthesis in LP diets. This might explain the high levels of Thr in blood plasma, as the excess Thr was not actively degraded to Gly. Fancher and Jensen (1989), observed an interesting effect of increased plasma Thr when reducing dietary CP. While this did not occur in the non-supplemented LP treatment, the supplementation of Thr in a LP diet disproportionately increased blood plasma free Thr compared to that seen in blood plasma free Gly

and Ser in Gly and Ser supplemented treatments. Furthermore, to facilitate the conversion of Gly to Ser by serine hydroxymethyltransferase a one carbon unit is required from N^5-N^{10}-methylenetetrahydrofolate. However, 50% of N^5-N^{10}-methylene tetrahydrofolate is produced from the Gly cleavage system, a Gly degradation metabolic pathway (Lamers et al., 2007). Therefore, the conversion of Gly to Ser should be assumed to be on closer to a 1.5:1.0 basis rather than the assumed 1.0:1.0 interconversion ratio. This could explain why a greater difference in blood plasma Gly and Ser occurred in the Ser supplemented diet compared to the Gly supplemented diet as more Gly was required to synthesize Ser.

Corzo et al. (2009), Ospina-Rojas et al. (2013a), and Siegert et al. (2015) all concluded that some Gly requirement can be overcome with slight increases in dietary Thr. This study suggests that the complete replacement of Gly with excess Thr negatively impacts performance when there is no deficiency of Gly_{equiv}. This study assumed that standard Thr requirements do not satisfy any Gly_{equiv} requirements. Edmonds and Baker (1987), saw that excess Thr significantly decreased FCR, feed intake, and body weight gain in young pigs which was not observed in this study with meat chickens. Choline and Thr degradation makes up $\leq$ 6% of Gly synthesis in young pigs, while dietary Ser makes up $\leq$ 7% of total Gly synthesis (Wang et al., 2013), accounting for only a fraction of Gly_{equiv} requirements. The fings of this study support that a similar ratio exists in chicken for Thr and that the supplementation of excess Thr fails to completely replace Gly_{equiv} requirements to achieve maximum efficient growth in LP diets. This implies that avian Gly metabolism differs from Gly metabolism in pigs. Further research into Gly sources in birds is required to gain a better understanding of LP diets and Gly_{equiv} requirements in broiler nutrition.

CONCLUSION

The Gly_{equiv} levels investigated in this experiment were not limiting and did not cause deterioration in performance. However, AA digestibility, SUA levels, and blood plasma AA concentrations were all affected by the supplementation of either Gly, Ser, or Thr, indicating changes in metabolic processes. Further research is required into the nutrients present in a SP diet containing MBM such as DEB, other AA, and phytase activity. Supplementing excess Thr suppressed performance when Gly_{equiv} was not deficient. Equimolar supplementation of Ser produced similar results to Gly, offering a possible alternative to Gly_{equiv} supplementation. The interactions between AA *in vivo* are complex and are influenced by many environmental and genetic variables. In order to better understand meat chicken nutrition fed LP diets, these interactions need to be comprehensively researched. The effect of reducing CP on varying Gly_{equiv} requirements with relation to CP constructs a difficult problem for solving precise Gly_{equiv} nutrition in LP diets.

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