

Influence of increasing glycine concentrations in reduced crude protein diets fed to broilers from 0 to 48 days

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ABSTRACT Two experiments investigated broiler growth performance and processing characteristics when fed increasing Gly concentrations in reduced CP diets fed from 0 to 48 d. In experiment 1, birds were allocated to 1 of 4 dietary treatments: a control (**CTL**) diet containing feed-grade L-Met, L-Lys, and L-Thr, a reduced CP (**RCP**) diet with additions of feed-grade L-Val and L-Ile, or the RCP diet with moderate (**M Gly**) or high Gly (**H Gly**) inclusion levels to achieve a total Gly + Ser of 100 or 112%, respectively, of the CTL diet. Birds in experiment 2 were assigned to 1 of 6 dietary treatments: a CTL diet, a RCP diet, or a low CP (**LCP**) diet without or with added Gly to achieve 88, 100, 112, or 124% total Gly + Ser concentrations of the RCP diet. For experiment 1, 0 to 14 d broiler performance was similar ($P > 0.05$) among dietary treatments. From 0 to 48

d, broilers fed the H Gly diet had the lowest ($P = 0.006$) body weight gain (**BWG**) and highest ($P = 0.003$) feed conversion ratio (**FCR**). Feeding either the RCP or M Gly diet resulted in similar ($P > 0.05$) growth and processing characteristics to the CTL. For experiment 2, increasing Gly levels in the LCP diet linearly reduced ($P \leq 0.027$) 0 to 14 d FI and FCR. From 0 to 48 d, broilers had similar ($P > 0.05$) performance when fed the CTL or RCP diet, but had a higher ($P < 0.001$) FCR when fed the LCP88 diet. Increasing Gly levels linearly reduced ($P = 0.033$) FCR. Total breast meat yield was negatively affected ($P \leq 0.020$) when feeding the LCP88 diet and did not respond to Gly levels. In conclusion, effects of increasing total Gly + Ser levels on 0 to 48 d broiler performance are likely dependent on the content of dietary CP and other potentially interacting nutrients.

Key words: feed-grade amino acid, glycine, serine, reduce protein, broiler

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INTRODUCTION

Researchers continue to focus on quantifying the extent that dietary crude protein (**CP**) can be reduced in order to achieve lower diet costs, improve bird health, and contribute to environmental sustainability while maintaining bird performance and processing yields. Dietary CP reductions can be accomplished by using individual feed-grade essential amino acids (**AA**) to help meet digestible essential AA minimums. Today, broader availability and reduced costs of other feed-grade essential AA beyond Met, Lys, and Thr, including Val, Ile, and Arg, have made it possible to further reduce CP in commercial settings. However, CP reductions have been shown to deteriorate broiler growth performance and meat yield due to one or more limiting nutrients (Bregendahl et al., 2002). Indeed,

it is commonly proposed that total Gly + Ser (**tGly + Ser**) concentration may be limiting when broilers are fed reduced CP, all-vegetable diets (Corzo et al., 2004; Dean et al., 2006).

Six experiments by Dean et al. (2006) demonstrated that increasing dietary tGly + Ser was the most effective among other potentially-limiting essential or specific nonessential AA in restoring performance of young (0 to 18 d) broilers fed reduced CP diets (16–22% CP diets). Others have also found that young (0 to 21 d) broilers need a minimum amount of tGly + Ser in the diet to sustain performance when fed reduced CP diets (Ngo et al., 1977; Corzo et al., 2004, 2005, Waguespack et al., 2009a, b; Yuan et al., 2012; Ospina-Rojas et al., 2013b; Kriseldi et al., 2017). However, maintaining suggested dietary tGly + Ser levels has failed to fully restore performance when compared to broilers fed recommended or higher CP level diets in some studies (Jiang et al., 2005; Waldroup et al., 2005). Recent research has suggested that additional nutrients are often equally or more limiting in reduced CP diets even when tGly + Ser levels are at or above estimated requirements (Belloir et al., 2017; Hilliar et al., 2019; Hofmann et al., 2019). This is likely

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due to the fact that tGly + Ser needs are influenced by its interactions with Met, Cys, Thr, Arg, Gln, Asp, CP, choline, guanidinoacetic acid, creatine, and betaine (Siegert et al., 2015; Belloir et al., 2017; Siegert and Rodehutschord, 2019). Furthermore, few studies have evaluated if the relevant improvements often observed in young broiler performance by restoring or increasing dietary tGly + Ser levels positively influence cumulative performance at market age and processing characteristics, with work being limited to Berres et al. (2010) and Kriseldi et al. (2017). In addition, there is currently a paucity of information determining the tGly + Ser requirements for older broilers.

Work by Kriseldi et al. (2017) found that feeding reduced CP diets (0 to 14 d: 24–22% CP) with added Gly to supply 11% higher tGly + Ser (2.14%, starter) levels was effective in maintaining broiler feed conversion ratio (**FCR**) and body weight gain (**BWG**) during 0 to 14 d and 15 to 28 d periods. However, for older broilers (28 to 40 d), FCR was improved with the addition of L-Gln, and not Gly. Cumulatively from 0 to 40 d, increasing tGly + Ser by 6% (2.03% tGly + Ser, starter), but not 11%, was more effective in restoring FCR than Gln supplementation with dietary CP reductions (2.4% units). Berres et al. (2010) observed that the combination of both feed-grade Gly and Glu, and not Gly alone, in reduced CP diets containing feed-grade L-Val and L-Ile (0 to 7 d: 26–24% CP) improved 0 to 42 d broiler performance. On the other hand, Gly supplementation alone improved 0 to 21 d FCR. As nutritionist implement more and higher inclusion levels of feed-grade AA, the reduction of intact proteins can cause conditionally essential and non-essential AA, such as Gly, to become potentially limiting for young and possibly older broilers. Given the lack of published work evaluating tGly + Ser needs for older broilers, it is evident that additional work is needed to evaluate the impact of tGly + Ser through market weight age and its effect on processing characteristics. Therefore, the objective of these experiments was to evaluate increasing tGly + Ser levels in reduced CP, corn-soybean meal-based diets fed to broilers from placement to market weight age at different degrees of CP reduction and determine the impact on growth performance and meat yield. It was hypothesized that Gly supplementation will be required for broilers to maintain performance and meat yield when fed reduced CP diets up to market weight age.

MATERIALS AND METHODS

All procedures involving live birds were approved by University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

General Procedure

Bird Husbandry For both experiments, male chicks from a Ross 708 female broiler breeder line (Aviagen North America, Sallisaw, OK) were received at hatch

and transported to the University of Arkansas poultry research farm. All birds were vaccinated in ovo for Marek's disease. Before placement, chicks were selected and weighed such that each group weight of chicks fell within 3% of the expected average based on a preliminary weight of approximately 20% of the population. Chicks were then placed in pens in a solid-sided research barn that was equipped with radiant tube heaters, vent boards, exhaust fans, and an electronic controller to maintain target environmental temperatures. Each pen contained one hanging feeder and a section (5 nipples/pen) of a continuous nipple drinker line that extended through each row of pens. Birds had ad libitum access to feed and water for the duration of the experiment. Ambient temperature at chick placement was set at 33°C and gradually decreased to maintain bird comfort as they aged until a final set point of 20°C was reached. The photoperiod was set at 23L:1D from placement to 7 d, 16L:8D from 8 to 28 d, and 18L:6D from 29 d until the end of the experiment. Light intensity was set at 27 lux from 1 to 7 d, 16 lux from 8 to 14 d, and 1 lux from 15 d to the end of the trial.

Dietary Treatments In both experiments dietary treatments were maintained across feeding phases, which consisted of starter (0 to 14 d), grower (14 to 28 d), finisher (28 to 39 d), and withdrawal (39 to 48 d) phases. Starter diets were pelleted and then crumbled, and grower, finisher, and withdrawal diets were pelleted. Prior to formulation, samples of corn and soybean meal were analyzed for total AA profile and CP content by wet chemistry analysis. All nutrient requirements, excluding CP, were formulated to meet or exceed primary breeder recommendations (Aviagen, 2019) and to be isocaloric with the same minimum digestible AA to digestible Lys ratios within each phase. All basal diets were mixed in a one-ton, vertical mixer prior to being pelleted. Complete experimental feeds from each growth phase were sampled and analyzed for nutrient composition.

Growth Performance Birds and feed were weighed at placement and 14, 28, 39, and 48 d to determine BWG, feed intake (**FI**), and mortality corrected FCR.

Plasma Amino Acids For experiment 2 on d 14 and 48, 1 randomly selected bird per pen from each of the LCP groups (9 birds/treatment) was euthanized by CO₂ and subjected to cardiac puncture for blood collection into tubes containing EDTA. After centrifuging, plasma was stored at –80°C until AA analysis. At time of analysis, plasma samples were deproteinized by mixing approximately 200 μ L of plasma with 100 μ L of DL-2-aminobutyric acid and 700 μ L acetonitrile (VWR Scientific, Radnor, PA). The mixture was centrifuged at 15,000 \times *g* for 15 min. Then 10 μ l of each supernatant and AA standard were derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivative kit (Waters Corp., Milford, MA). The derivatization of the AA was separated by an Acquity H-Class ultra-performance liquid chromatography instrument (Waters Corp.) with fluorescent detector and the AccQ-Tag Ultra C-18 column (2.1 mm \times 100 mm, 1.7 μ m; Waters

Corp.). Waters Empower chromatography software was used to control system, collect, and process data.

Processing For each experiment on d 48, after all birds were weighed by pen, 6 birds per pen (experiment 1: 288; experiment 2: 360 birds) were randomly selected for processing and wing-banded. On d 49, following 10 h of feed removal, banded birds were transported to the University of Arkansas Pilot Processing Plant and weighed individually prior to processing. Birds were humanely electrically stunned prior to exsanguination via jugular vein incision. Thereafter, birds were scalded and defeathered and the neck, heads, and feet were removed. After hand-removal of the viscera and the lungs, hot carcass and fat pad weights were recorded. Hot carcasses were submerged in an ice water bath for 4 h, and chilled carcasses were weighed and manually deboned to collect weights of breast fillets, tenders, wings, and leg quarters. Percentage yield of each of these parts was determined relative to individual back dock live BW.

Experiment 1

A total of 1,008 chicks were allocated to 48 floor pens (0.09 m²/bird; 21 birds/pen) with unused pine shavings as bedding. Chicks were provided 1 of 4 dietary treatments within a block of pens, with 12 replicate pens per treatment (Tables 1 and 2). Four dietary treatments included a control (CTL) diet with feed-grade L-Met, L-Lys, and L-Thr, a reduced CP (RCP) diet that included feed-grade L-Val and L-Ile, and the RCP diet with added feed-grade Gly at 2 concentrations (moderate [M] or high [H] Gly). For each diet phase, the M Gly diet had a tGly + Ser concentration that was equal to the CTL diet (e.g., starter: 2.17%) while the H Gly diet had the highest tGly + Ser concentration (e.g., starter: 2.46%) among the dietary treatments, and these relative difference were maintained across each phase.

Experiment 2

A total of 1,260 chicks were allocated to 60 floor pens (0.09 m²/bird; 21 [treatments 1-2] or 22 [treatments 3-6] birds/pen) with a bedding of de-caked litter top-dressed with fresh pine shavings. Chicks were allocated to 1 of 6 dietary treatments within a block of pens, with 12 (treatments 1-2) or 9 (treatments 3-6) replicate pens per treatment (Tables 3-6). Dietary treatments included a CTL similar to experiment 1, a reduced CP (RCP) diet with feed-grade L-Val and L-Ile in all phases and L-Arg from 28 to 48 d, and four low CP (LCP) diets with additions of L-Arg at every phase and L-Trp from 39 to 48 d with added supplemental Gly to achieve 88 (no added Gly; LCP88), 100 (LCP100), 112 (LCP112), or 124% (LCP124) tGly + Ser concentrations of the RCP diet. For the mixing of the LCP diets, LCP88 was considered the basal and LCP124 the summit diet and these diets were blended to create the LCP100 and LCP 112 diets, respectively.

Statistical Analysis

For experiments 1 and 2, dietary treatments were arranged in a randomized complete block design and pen location served as the blocking factor. For experiment 1, data were analyzed by one-way ANOVA using MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC). For experiment 2, data were analyzed with a pre-planned orthogonal contrast and conducted to investigate 3 primary research questions: CTL vs. RCP, CTL vs. LCP88, and RCP vs. LCP88. In addition, linear and quadratic contrasts were used to examine the effects of increasing the tGly + Ser concentration in the LCP88 diet. The SEM reported for experiment 2 are only from the LCP group due to the lower number of replications resulting in minor differences in SEM values than the CTL and RCP groups. Furthermore, reporting only the LCP group SEM was done to improve simplicity and readability of the tables. Statistical significance was considered at $P \leq 0.05$ in all cases. When appropriate for experiment 1 data, means were separated using Tukey's multiple comparison test. Mortality were subjected to a square root arcsine transformation prior to analysis.

RESULTS

Experiment 1

Diet Analyses The inclusion of L-Val and L-Ile reduced overall analyzed CP by an average of 1.68% units (calculated 1.92% units) and tGly + Ser by 0.21% units (calculated 0.18% units; Tables 1 and 2). Relative to the RCP diet, average increases in analyzed tGly + Ser for the M Gly and H Gly were 0.18 and 0.31% units (calculated 0.18 and 0.41% units), respectively. All other analyzed AA were in close agreement with calculated values with an average difference (calculated vs. analyzed total AA) of 4.81% for all AA and an average minimum and maximum difference of 0.41% and 9.00%, respectively, with the exception of TSAA having slightly larger range than expected (15.71–24.63% difference).

0 to 14 d Performance Reducing dietary CP did not impact ($P > 0.05$) BWG, FI, or FCR during the starter phase (Table 7). Increasing the tGly + Ser in the RCP diet to equal (M Gly diet) or higher (H Gly diet) tGly + Ser levels of the CTL diet did not affect ($P > 0.05$) chick starter performance.

0 to 28 d Performance Birds fed the CTL or RCP diet had similar ($P > 0.05$) BWG, FI, and FCR. Increasing tGly + Ser in the M Gly diet did not affect bird BWG, FI, or FCR, whereas further supplementation of Gly (H Gly) did not affect FI ($P = 0.177$) but reduced BWG ($P = 0.015$) and increased FCR ($P = 0.003$) when compared to birds fed the CTL or M Gly diet. However, bird BWG, FI, and FCR were similar to RCP-fed when fed either the M Gly or H Gly diet.

0 to 39 d Performance Broilers fed the CTL, RCP, or M Gly diet had similar ($P > 0.05$) BWG, FI, and FCR. For broilers fed the H Gly diet, BWG ($P = 0.003$) and FI ($P = 0.009$) were the lowest among all treatment

Table 1. Ingredient, calculated, and analyzed composition (% , unless otherwise noted) of experiment 1 diets fed to broilers from 0 to 14 d and 14 to 28 d post-hatch.

Ingredient, as-fed	0 to 14 d				14 to 28 d			
	CTL	RCP	M Gly	H Gly	CTL	RCP	M Gly	H Gly
Corn	50.91	57.53	57.18	56.72	56.00	61.71	61.41	61.01
Soybean meal (47%)	42.42	36.28	36.32	36.36	36.78	31.61	31.64	31.68
Poultry fat	3.28	2.09	2.17	2.29	4.04	2.97	3.05	3.15
Limestone	1.08	1.11	1.11	1.11	1.03	1.06	1.06	1.05
Dicalcium phosphate	1.11	1.13	1.14	1.14	0.90	0.92	0.92	0.92
Sodium chloride	0.33	0.12	0.12	0.12	0.30	0.25	0.25	0.25
L-Met	0.32	0.37	0.37	0.37	0.30	0.34	0.34	0.34
L-Lys-HCl	0.07	0.25	0.25	0.25	0.07	0.22	0.22	0.22
L-Thr	0.13	0.21	0.21	0.21	0.11	0.18	0.18	0.18
L-Val	-	0.09	0.09	0.09	-	0.08	0.08	0.08
L-Ile	-	0.08	0.08	0.08	-	0.07	0.07	0.07
Glycine	-	-	0.22	0.52	-	-	0.19	0.45
Vitamin premix ¹	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix ²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Se premix ³ (0.06%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (60%)	0.04	0.06	0.06	0.06	0.03	0.05	0.05	0.05
Sodium bicarbonate	0.02	0.37	0.37	0.37	0.15	0.24	0.24	0.24
Copper chloride ⁴ (54%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Cocciostat ⁵	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase ⁶	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient composition								
AME _n , kcal/kg	3,000	3,000	3,000	3,000	3,100	3,100	3,100	3,100
CP	24.11	22.07	22.38	22.66	21.80	20.10	20.32	20.16
Digestible Lys	1.28	1.28	1.28	1.28	1.15	1.15	1.15	1.15
Digestible Met	0.64	0.66	0.66	0.66	0.59	0.61	0.61	0.61
Digestible TSAA	0.95	0.95	0.95	0.95	0.87	0.87	0.87	0.87
Met:Cys ratio	67:33	69:31	69:31	69:31	68:32	70:30	70:30	70:30
Digestible Thr	0.86	0.86	0.86	0.86	0.77	0.77	0.77	0.77
Digestible Val	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87
Digestible Ile	0.88	0.86	0.86	0.86	0.79	0.78	0.78	0.78
Digestible Arg	1.55	1.37	1.37	1.37	1.38	1.23	1.23	1.23
Digestible Trp	0.24	0.21	0.21	0.21	0.21	0.19	0.19	0.19
Total Gly + Ser	2.17	1.95	2.17	2.46	1.96	1.77	1.96	2.21
Total Ca	0.96	0.96	0.95	0.96	0.87	0.87	0.87	0.87
Available P	0.48	0.48	0.48	0.48	0.44	0.44	0.44	0.44
Choline, mg/kg	1,900	1,900	1,900	1,900	1,750	1,750	1,750	1,750
dEB, mEq/kg	295	295	295	295	280	256	256	255
Analyzed nutrient composition								
CP	25.18	23.31	22.09	24.24	22.19	21.26	20.23	20.22
Total Lys	1.49	1.46	1.38	1.48	1.33	1.36	1.29	1.28
Total TSAA	1.15	1.18	1.23	1.29	1.12	1.10	1.14	1.18
Total Thr	1.10	1.07	1.04	1.04	0.94	0.95	0.92	0.92
Total Val	1.23	1.18	1.13	1.18	1.08	1.10	1.03	1.02
Total Ile	1.09	1.06	0.96	1.04	0.93	0.93	0.88	0.86
Total Leu	2.08	1.89	1.78	1.87	1.80	1.73	1.64	1.57
Total Arg	1.40	1.29	1.23	1.41	1.30	1.18	1.14	1.15
Total Trp	0.33	0.29	0.27	0.31	0.30	0.28	0.26	0.25
Total Gly + Ser	2.38	2.13	2.23	2.56	2.07	1.97	2.05	2.20
Gly _{equi}	2.00	1.79	1.91	2.22	1.74	1.66	1.75	1.92

Abbreviations: AME_n, nitrogen-corrected apparent metabolizable energy; CTL, control; dEB, dietary electrolyte balance; H, high; M, moderate; RCP, reduced crude protein.

¹The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin D₃, 4,536 ICU; vitamin E, 45 IU; vitamin B₁₂ 0.01 mg; menadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

²The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

³Supplied 0.12 mg of selenium per kg of diet.

⁴IntelliBond C, Micronutrients, Indianapolis, IN.

⁵Salinomycin sodium, Bio-Cox, Huvepharma Inc., Peachtree City, GA.

⁶Optiphos, Huvepharma Inc., Peachtree City, GA.

groups, although FI of the H Gly-birds was similar to the CTL-fed. FCR of broilers fed the H Gly was higher ($P = 0.001$) than that of the CTL and RCP-fed broilers but similar to the M Gly-fed broilers.

0 to 48 d Performance Reducing dietary CP resulted in similar ($P > 0.05$) broiler BWG, FI, and FCR to broilers fed the CTL diet. Feeding the M Gly diet had equal ($P > 0.05$) BWG, FI, and FCR compared to broilers fed the CTL or RCP diet. Broilers fed the H Gly

diet had the lowest BWG ($P = 0.006$) and highest FCR ($P = 0.003$), but comparable FI ($P = 0.074$) among the treatment groups. FCR was similar between broilers fed the RCP and M Gly diets. The period and cumulative mortality were unaffected ($P > 0.05$) by treatments (data not shown). Comparing to breeder performance objectives, 0 to 48 d average BWG of 3.631 kg and FCR of 1.568 are in accordance with objectives (3.622 kg and 1.692, respectively).

Table 2. Ingredient, calculated, and analyzed composition (% , unless otherwise noted) of experiment 1 diets fed to broilers from 28 to 39 d and 39 to 48 d post-hatch.

Ingredient, as-fed	28 to 39 d				39 to 48 d			
	CTL	RCP	M Gly	H Gly	CTL	RCP	M Gly	H Gly
Corn	60.95	65.84	65.58	65.26	64.52	68.46	68.25	67.97
Soybean meal (47%)	31.37	26.95	26.98	27.01	28.58	25.02	25.04	25.07
Poultry fat	4.79	3.89	3.95	4.03	4.16	3.43	3.48	3.55
Limestone	0.98	1.00	1.00	1.00	0.97	0.99	0.99	0.99
Dicalcium phosphate	0.68	0.70	0.70	0.70	0.61	0.62	0.62	0.63
Sodium chloride	0.30	0.25	0.26	0.26	0.30	0.26	0.26	0.26
L-Met	0.27	0.30	0.30	0.30	0.24	0.27	0.27	0.27
L-Lys-HCl	0.07	0.20	0.20	0.20	0.07	0.18	0.18	0.18
L-Thr	0.10	0.16	0.16	0.16	0.09	0.14	0.14	0.14
L-Val	-	0.05	0.05	0.05	-	0.05	0.05	0.05
L-Ile	-	0.07	0.07	0.07	-	0.06	0.06	0.06
Glycine	-	-	0.17	0.38	-	-	0.13	0.32
Vitamin premix ¹	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix ²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Se premix ³ (0.06%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (60%)	0.03	0.04	0.04	0.04	0.02	0.04	0.04	0.04
Sodium bicarbonate	0.16	0.24	0.24	0.24	0.17	0.23	0.23	0.23
Copper chloride ⁴ (54%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Cocciostat ⁵	0.05	0.05	0.05	0.05	-	-	-	-
Phytase ⁶	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient composition								
AME _n , kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
CP	19.58	18.11	18.31	18.54	18.51	17.34	17.49	17.69
Digestible Lys	1.02	1.02	1.02	1.02	0.96	0.96	0.96	0.96
Digestible Met	0.53	0.55	0.55	0.55	0.49	0.51	0.51	0.51
Digestible TSAA	0.80	0.80	0.80	0.80	0.75	0.75	0.75	0.75
Met:Cys ratio	66:34	69:31	69:31	69:31	65:35	68:32	68:32	68:32
Digestible Thr	0.68	0.68	0.68	0.68	0.64	0.64	0.64	0.64
Digestible Val	0.79	0.78	0.78	0.78	0.75	0.75	0.75	0.75
Digestible Ile	0.70	0.70	0.70	0.70	0.66	0.66	0.66	0.66
Digestible Arg	1.22	1.09	1.09	1.09	1.14	1.04	1.04	1.04
Digestible Trp	0.22	0.19	0.19	0.19	0.18	0.16	0.16	0.16
Total Gly + Ser	1.76	1.59	1.76	1.96	1.66	1.53	1.66	1.84
Total Ca	0.78	0.78	0.78	0.78	0.75	0.75	0.75	0.75
Available P	0.39	0.39	0.39	0.39	0.38	0.38	0.38	0.38
Choline, mg/kg	1,650	1,650	1,650	1,650	1,600	1,600	1,600	1,600
dEB, mEq/kg	254	233	232	232	241	224	224	224
Analyzed nutrient composition								
CP	20.54	18.02	19.18	18.37	18.99	17.58	17.90	17.74
Total Lys	1.14	1.03	1.08	1.06	1.09	1.09	1.03	1.03
Total TSAA	1.23	1.10	1.16	1.05	1.09	1.14	1.15	1.20
Total Thr	0.81	0.74	0.79	0.78	0.78	0.77	0.75	0.75
Total Val	1.02	0.94	1.00	0.96	0.93	0.90	0.91	0.91
Total Ile	0.97	0.93	0.95	0.92	0.77	0.76	0.88	0.88
Total Leu	1.72	1.57	1.62	1.60	1.54	1.45	1.57	1.52
Total Arg	1.00	0.79	0.94	0.95	1.13	0.97	0.80	0.87
Total Trp	0.27	0.22	0.22	0.22	0.24	0.22	0.22	0.22
Total Gly + Ser	1.83	1.52	1.79	1.89	1.76	1.60	1.63	1.81
Gly _{equi}	1.54	1.28	1.53	1.64	1.48	1.35	1.39	1.57

Abbreviations: AME_n, nitrogen-corrected apparent metabolizable energy; CTL, control; dEB, dietary electrolyte balance; H, high; M, moderate; RCP, reduced crude protein.

¹The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin D₃, 4,536 ICU; vitamin E, 45 IU; vitamin B₁₂ 0.01 mg; menadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

²The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

³Supplied 0.12 mg of selenium per kg of diet.

⁴IntelliBond C, Micronutrients, Indianapolis, IN.

⁵Salinomycin sodium, Bio-Cox, Huvepharma Inc., Peachtree City, GA.

⁶Optiphos, Huvepharma Inc., Peachtree City, GA.

Processing Hot and chilled carcass weight ($P < 0.05$) and yield ($P < 0.05$) were highest for the CTL group, intermediate for the RCP and M Gly groups, and lowest for the H Gly group (Table 8). Absolute ($P = 0.017$) and relative ($P = 0.007$) fat pad weights were lowest for the CTL group, intermediate for the RCP group, and highest for M Gly group. The absolute fat pad weight of the H Gly group was intermediate, whereas relative weight was similar to the M

Gly and RCP groups. For deboned parts, breast fillet, total breast meat, and wing weight and yield were not affected ($P > 0.05$) by diet. Tender weight ($P = 0.007$), but not yield ($P > 0.05$), was lowest for the H Gly group, intermediate for M Gly, and highest for the CTL and RCP groups. Leg quarter weight ($P = 0.003$) and yield ($P = 0.025$) were lowest for the H Gly group, intermediate for the RCP and M Gly groups, and highest for the CTL group.

Table 3. Ingredient, calculated, and analyzed nutrient composition (% , unless otherwise noted) of experiment 2 starter diets fed to broilers from 0 to 14 d post-hatch.

Ingredient, as-fed	CTL	RCP	LCP88 ¹	LCP100	LCP112	LCP124
Corn	55.85	58.05	65.34	64.99	64.63	64.27
Soybean meal (48%)	38.03	36.00	29.24	29.24	29.24	29.24
Poultry fat	2.50	2.10	0.70	0.80	0.89	0.98
Limestone	1.10	1.12	1.15	1.15	1.15	1.15
Dicalcium phosphate	1.13	1.13	1.16	1.17	1.17	1.17
Sodium chloride	0.28	0.25	0.18	0.18	0.18	0.18
L-Met	0.31	0.33	0.39	0.39	0.39	0.39
L-Lys·HCl	0.13	0.20	0.41	0.41	0.41	0.41
L-Thr	0.12	0.15	0.24	0.24	0.24	0.24
L-Val	-	0.03	0.14	0.14	0.14	0.15
L-Ile	-	0.01	0.12	0.12	0.12	0.12
L-Arg	-	-	0.19	0.19	0.19	0.20
Glycine	-	-	-	0.26	0.51	0.77
Cellulose ²	-	0.05	-	-	-	-
Vitamin premix ³	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix ⁴	0.10	0.10	0.10	0.10	0.10	0.10
Se premix ⁵ (0.06%)	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (60%)	0.06	0.06	0.09	0.09	0.09	0.09
Sodium bicarbonate	0.18	0.21	0.33	0.33	0.33	0.33
Copper chloride ⁶ (54%)	0.06	0.06	0.09	0.09	0.09	0.09
Coccidiostat ⁷	0.05	0.05	0.05	0.05	0.05	0.05
Phytase ⁸	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient composition						
AME _n , kcal/kg	3,000	3,000	3,000	3,000	3,000	3,000
CP	22.86	22.20	19.92	20.18	20.44	20.70
Digestible Lys	1.28	1.28	1.28	1.28	1.28	1.28
Digestible Met	0.66	0.67	0.70	0.70	0.70	0.70
Digestible TSAA	0.95	0.95	0.95	0.95	0.95	0.95
Met:Cys ratio	69:31	71:29	74:26	74:26	74:26	74:26
Digestible Thr	0.86	0.86	0.86	0.86	0.86	0.86
Digestible Val	0.96	0.96	0.96	0.96	0.96	0.96
Digestible Ile	0.88	0.86	0.86	0.86	0.86	0.86
Digestible Arg	1.53	1.47	1.34	1.34	1.34	1.34
Digestible Trp	0.28	0.27	0.23	0.23	0.23	0.23
Total Gly + Ser	2.12	2.05	1.80	2.05	2.30	2.55
Total Ca	0.96	0.96	0.96	0.96	0.96	0.96
Available P	0.48	0.48	0.48	0.48	0.48	0.48
Choline, mg/kg	1,900	1,900	1,900	1,900	1,900	1,900
dEB, mEq/kg	288	278	246	245	245	245
Analyzed nutrient composition						
CP	23.56	22.31	21.75	-	-	-
Total Lys	1.43	1.50	1.59	1.46	1.40	1.57
Total TSAA	0.95	0.93	0.91	0.93	0.92	0.93
Total Thr	1.01	1.00	0.99	0.95	0.96	0.98
Total Val	1.11	1.13	1.11	1.08	1.06	1.12
Total Ile	0.99	0.99	0.98	0.94	0.93	0.98
Total Leu	1.95	1.94	1.82	1.73	1.72	1.79
Total Arg	1.56	1.49	1.48	1.40	1.41	1.48
Total Trp	0.31	0.30	0.27	0.26	0.25	0.27
Total Gly + Ser	2.14	2.05	1.87	2.01	2.24	2.50
Gly _{equi}	1.81	1.73	1.58	1.74	1.97	2.21

Abbreviations: AME_n, nitrogen-corrected apparent metabolizable energy; CTL, control; dEB, dietary electrolyte balance; LCP, low crude protein.

¹For the mixing of the LCP diets, LCP88 was considered the basal and LCP124 the summit diet and these diets were blended to create the LCP100 and LCP 112 diets.

²The inclusion of cellulose in the RCP diet was formulated for use in a separate experiment as an inert filler, and served no relevant purpose in this study.

³The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin D₃, 4,536 ICU; vitamin E, 45 IU; vitamin B₁₂ 0.01 mg; menadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

⁴The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

⁵Supplied 0.12 mg of selenium per kg of diet.

⁶IntelliBond C, Micronutrients, Indianapolis, IN.

⁷Salinomycin sodium, Bio-Cox, Huvepharma Inc., Peachtree City, GA.

⁸Optiphos, Huvepharma Inc., Peachtree City, GA.

Experiment 2

Diet Analyses The inclusion of L-Val and L-Ile (all phases) and L-Arg (finisher and withdrawal phases) in the RCP diet lowered analyzed dietary CP by an average of 0.64% units (calculated 1.04% units) and

tGly + Ser by 0.09% units (0.12% units calculated; Tables 3–6). Further CP reduction in the LCP diet with the addition of L-Arg (every phase) and L-Trp (finisher phase) resulted in an average analyzed reduction in CP by 2.38% units (calculated 2.96% units) and tGly + Ser by 0.24% units (calculated 0.21% units).

Table 4. Ingredient, calculated, and analyzed nutrient composition (% , unless otherwise noted) of experiment 2 grower diets fed to broilers from 14 to 28 d post-hatch.

Ingredient, as-fed	CTL	RCP	LCP88 ¹	LCP100	LCP112	LCP124
Corn	60.68	62.28	68.32	68.00	68.00	67.68
Soybean meal (48%)	32.72	31.23	25.34	25.34	25.34	25.34
Poultry fat	3.28	2.98	1.76	1.84	1.92	2.01
Limestone	1.03	1.04	1.07	1.07	1.07	1.07
Dicalcium phosphate	0.94	0.94	0.97	0.97	0.97	0.98
Sodium chloride	0.27	0.26	0.20	0.20	0.20	0.20
L-Met	0.29	0.30	0.35	0.35	0.35	0.36
L-Lys·HCl	0.14	0.19	0.37	0.37	0.37	0.37
L-Thr	0.11	0.13	0.20	0.20	0.21	0.21
L-Val	-	0.02	0.12	0.12	0.12	0.12
L-Ile	-	0.02	0.11	0.11	0.11	0.11
L-Arg	-	-	0.17	0.17	0.17	0.17
Glycine	-	-	-	0.23	0.46	0.69
Cellulose ²	-	0.04	-	-	-	-
Vitamin premix ³	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix ⁴	0.10	0.10	0.10	0.10	0.10	0.10
Se premix ⁵ (0.06%)	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (60%)	0.04	0.05	0.07	0.07	0.07	0.07
Sodium bicarbonate	0.19	0.22	0.32	0.32	0.32	0.32
Copper chloride ⁶ (54%)	0.02	0.02	0.02	0.02	0.02	0.02
Coccidiostat ⁷	0.05	0.05	0.05	0.05	0.05	0.05
Phytase ⁸	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient composition						
AME _n , kcal/kg	3,100	3,100	3,100	3,100	3,100	3,100
CP	20.65	20.17	18.19	18.44	18.68	18.93
Digestible Lys	1.15	1.15	1.15	1.15	1.15	1.15
Digestible Met	0.61	0.62	0.64	0.64	0.64	0.64
Digestible TSAA	0.87	0.87	0.87	0.87	0.87	0.87
Met:Cys ratio	70:30	71:29	74:26	74:26	74:26	74:26
Digestible Thr	0.77	0.77	0.77	0.77	0.77	0.77
Digestible Val	0.87	0.87	0.87	0.87	0.87	0.87
Digestible Ile	0.79	0.78	0.78	0.78	0.78	0.78
Digestible Arg	1.25	1.21	1.21	1.21	1.21	1.21
Digestible Trp	0.25	0.24	0.20	0.20	0.20	0.20
Total Gly + Ser	1.92	1.87	1.65	1.87	2.10	2.32
Total Ca	0.87	0.87	0.87	0.87	0.87	0.87
Available P	0.44	0.44	0.44	0.44	0.44	0.44
Choline, mg/kg	1,750	1,750	1,750	1,750	1,750	1,750
dEB, mEq/kg	262	254	226	226	226	226
Analyzed nutrient composition						
CP	20.94	21.31	18.94	-	-	-
Total Lys	1.31	1.35	1.29	1.26	1.23	1.26
Total TSAA	0.82	0.83	0.85	0.87	0.90	0.86
Total Thr	0.92	0.96	0.87	0.90	0.81	0.86
Total Val	1.03	1.06	0.99	0.97	0.94	0.96
Total Ile	0.91	0.92	0.85	0.85	0.83	0.84
Total Leu	1.85	1.86	1.64	1.62	1.54	1.64
Total Arg	1.41	1.41	1.32	1.30	1.27	1.31
Total Trp	0.28	0.29	0.24	0.23	0.22	0.24
Total Gly + Ser	1.96	1.95	1.66	1.87	2.02	2.25
Gly _{equi}	1.65	1.64	1.40	1.61	1.78	1.99

Abbreviations: AME_n, nitrogen-corrected apparent metabolizable energy; CTL, control; dEB, dietary electrolyte balance; LCP, low crude protein; RCP, reduced crude protein.

¹For the mixing of the LCP diets, LCP88 was considered the basal and LCP124 the summit diet and these diets were blended to create the LCP100 and LCP 112 diets.

²The inclusion of cellulose in the RCP diet was formulated for use in a separate experiment as an inert filler, and served no relevant purpose in this study.

³The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin D₃, 4,536 ICU; vitamin E, 45 IU; vitamin B₁₂, 0.01 mg; menadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

⁴The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

⁵Supplied 0.12 mg of selenium per kg of diet.

⁶IntelliBond C, Micronutrients, Indianapolis, IN.

⁷Salinomycin sodium, Bio-Cox, Huvepharma Inc., Peachtree City, GA.

⁸Optiphos, Huvepharma Inc., Peachtree City, GA.

Analyzed tGly + Ser averaged 0.5% lower than calculated values among all diets. Calculated tGly + Ser was set at 88, 100, 112, and 124% in the LCP diets relative to the RCP diet but the averaged analyzed tGly + Ser was 86, 97, 106, and 119%, respectively. All other analyzed AA were in expected ranges with an average

difference (calculated vs. analyzed total AA) of -0.67% and an average minimum and maximum difference of -4.24% and 2.94%.

0 to 14 d Performance For the starter phase, reducing dietary CP (CTL vs. RCP) did not affect ($P > 0.05$) chick BWG or FI (Table 9). Chicks fed the LCP88 diet

Table 5. Ingredient, calculated, and analyzed composition (% , unless otherwise noted) of experiment 2 finisher diets fed to broilers from 28 to 39 d post-hatch.

Ingredient, as-fed	CTL	RCP	LCP88 ¹	LCP100	LCP112	LCP124
Corn	65.44	70.26	75.62	75.35	75.08	74.81
Soybean meal (48%)	27.43	22.88	17.79	17.79	17.79	17.79
Poultry fat	4.08	3.16	2.17	2.23	2.30	2.37
Limestone	0.99	1.02	1.04	1.04	1.04	1.04
Dicalcium phosphate	0.70	0.72	0.74	0.74	0.74	0.75
Sodium chloride	0.27	0.23	0.06	0.06	0.06	0.06
L-Met	0.26	0.30	0.34	0.34	0.34	0.34
L-Lys·HCl	0.14	0.29	0.45	0.45	0.45	0.45
L-Thr	0.09	0.15	0.22	0.22	0.22	0.22
L-Val	-	0.06	0.14	0.14	0.15	0.15
L-Ile	-	0.07	0.16	0.16	0.16	0.16
L-Arg	-	0.12	0.27	0.27	0.27	0.27
Glycine	-	-	-	0.19	0.38	0.57
Cellulose ²	-	0.05	-	-	-	-
Vitamin/Mineral premix ^{3,4}	0.25	0.25	0.25	0.25	0.25	0.25
Se premix ⁵ (0.06%)	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (60%)	0.04	0.06	0.07	0.07	0.08	0.08
Sodium bicarbonate	0.21	0.29	0.59	0.59	0.59	0.59
Copper chloride ⁶ (54%)	0.02	0.02	0.02	0.02	0.02	0.02
Cocciostat ⁷	0.05	0.05	0.05	0.05	0.05	0.05
Phytase ⁸	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient composition						
AME _n , kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200
CP	18.44	16.94	15.21	15.41	15.62	15.83
Digestible Lys	1.02	1.02	1.02	1.02	1.02	1.02
Digestible Met	0.55	0.57	0.59	0.59	0.59	0.59
Digestible TSAA	0.80	0.80	0.80	0.80	0.80	0.80
Met:Cys ratio	69:31	71:29	74:26	74:26	74:26	74:26
Digestible Thr	0.68	0.68	0.68	0.68	0.68	0.68
Digestible Val	0.79	0.78	0.78	0.78	0.78	0.78
Digestible Ile	0.70	0.70	0.70	0.70	0.70	0.70
Digestible Arg	1.10	1.09	1.09	1.09	1.09	1.09
Digestible Trp	0.22	0.20	0.17	0.17	0.17	0.17
Total Gly + Ser	1.72	1.55	1.36	1.55	1.74	1.92
Total Ca	0.78	0.78	0.78	0.78	0.78	0.78
Available P	0.39	0.39	0.39	0.39	0.39	0.39
Choline, mg/kg	1,650	1,650	1,650	1,650	1,650	1,650
dEB, mEq/kg	235	213	213	213	213	213
Analyzed nutrient composition						
CP	19.06	18.38	16.31	-	-	-
Total Lys	1.18	1.14	1.14	1.15	1.15	1.16
Total TSAA	0.77	0.79	0.78	0.79	0.81	0.77
Total Thr	0.80	0.78	0.79	0.79	0.80	0.81
Total Val	0.93	0.89	0.86	0.87	0.86	0.87
Total Ile	0.80	0.80	0.76	0.75	0.76	0.76
Total Leu	1.75	1.62	1.42	1.47	1.46	1.46
Total Arg	1.21	1.22	1.18	1.19	1.20	1.19
Total Trp	0.24	0.22	0.19	0.19	0.19	0.19
Total Gly + Ser	1.72	1.59	1.35	1.57	1.74	1.95
Gly _{equi}	1.45	1.34	1.14	1.35	1.52	1.73

Abbreviations: AME_n, nitrogen-corrected apparent metabolizable energy; CTL, control; dEB, dietary electrolyte balance; LCP, low crude protein; RCP, reduced crude protein.

¹For the mixing of the LCP diets, LCP88 was considered the basal and LCP124 the summit diet and these diets were blended to create the LCP100 and LCP 112 diets.

²The inclusion of cellulose in the RCP diet was formulated for use in a separate experiment as an inert filler, and served no relevant purpose in this study.

³The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin D₃, 4,536 ICU; vitamin E, 45 IU; vitamin B₁₂ 0.01 mg; menadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

⁴The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

⁵Supplied 0.12 mg of selenium per kg of diet.

⁶IntelliBond C, Micronutrients, Indianapolis, IN.

⁷Salinomycin sodium, Bio-Cox, Huvepharma Inc., Peachtree City, GA.

⁸Optiphos, Huvepharma Inc., Peachtree City, GA.

had greater FI ($P < 0.001$) and BWG ($P \leq 0.024$) compared to chicks fed either the CTL or RCP diet, and both the RCP and LCP88-fed chicks had higher FCR ($P = 0.005$) than the CTL-fed chicks. Increasing tGly + Ser levels in the LCP diet linearly reduced FI

($P = 0.002$) and FCR ($P = 0.027$) without affecting BWG ($P > 0.05$).

0 to 28 d Performance Feeding the RCP diet did not affect ($P > 0.05$) bird BWG, FI, or FCR relative to the CTL-fed birds from 0 to 28 d. Birds fed the LCP88 diet

Table 6. Ingredient, calculated, and analyzed nutrient composition (% unless otherwise noted) of experiment 2 withdrawal diets fed to broilers from 39 to 48 d post-hatch.

Ingredient, as-fed	CTL	RCP	LCP88 ¹	LCP100	LCP112	LCP124
Corn	68.46	73.28	78.56	78.31	78.05	77.80
Soybean meal (48%)	25.18	20.63	15.64	15.64	15.64	15.64
Poultry fat	3.53	2.61	1.62	1.68	1.75	1.82
Limestone	0.97	1.00	1.02	1.02	1.02	1.02
Dicalcium phosphate	0.59	0.61	0.64	0.64	0.64	0.64
Sodium chloride	0.27	0.23	0.06	0.06	0.06	0.06
L-Met	0.23	0.27	0.31	0.31	0.31	0.31
L-Lys·HCl	0.14	0.28	0.44	0.44	0.44	0.44
L-Thr	0.08	0.14	0.20	0.21	0.21	0.21
L-Val	-	0.07	0.15	0.15	0.15	0.15
L-Ile	-	0.07	0.15	0.15	0.15	0.15
L-Arg	-	0.13	0.27	0.27	0.27	0.27
L-Trp	-	-	0.01	0.01	-	-
Glycine	-	-	-	0.18	0.36	0.55
Cellulose ²	-	0.04	-	-	-	-
Vitamin/Mineral premix ^{3,4}	0.25	0.25	0.25	0.25	0.25	0.25
Se premix ⁵ (0.06%)	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (60%)	0.04	0.05	0.07	0.07	0.07	0.07
Sodium bicarbonate	0.21	0.29	0.56	0.56	0.56	0.57
Copper chloride ⁶ (54%)	0.02	0.02	0.02	0.02	0.02	0.02
Phytase ⁷	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient composition						
AME _n , kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200
CP	17.55	16.05	14.36	14.55	14.75	14.95
Digestible Lys	0.96	0.96	0.96	0.96	0.96	0.96
Digestible Met	0.51	0.53	0.55	0.55	0.55	0.55
Digestible TSAA	0.75	0.75	0.75	0.75	0.75	0.75
Met:Cys ratio	68:32	71:29	73:27	73:27	73:27	73:27
Digestible Thr	0.64	0.64	0.64	0.64	0.64	0.64
Digestible Val	0.76	0.75	0.75	0.75	0.75	0.75
Digestible Ile	0.67	0.66	0.66	0.66	0.66	0.66
Digestible Arg	1.04	1.04	1.04	1.04	1.04	1.04
Digestible Trp	0.20	0.18	0.15	0.15	0.15	0.15
Total Gly + Ser	1.64	1.47	1.29	1.47	1.65	1.82
Total Ca	0.74	0.74	0.74	0.74	0.74	0.74
Available P	0.48	0.47	0.37	0.37	0.37	0.37
Choline, mg/kg	1,600	1,600	1,600	1,600	1,600	1,600
dEB, mEq/kg	225	203	200	200	200	200
Analyzed nutrient composition						
CP	18.19	17.19	15.25	-	-	-
Total Lys	1.08	1.07	1.04	1.03	1.01	1.05
Total TSAA	0.74	0.72	0.71	0.71	0.75	0.69
Total Thr	0.75	0.76	0.73	0.73	0.70	0.70
Total Val	0.87	0.84	0.79	0.79	0.81	0.80
Total Ile	0.76	0.74	0.67	0.67	0.69	0.69
Total Leu	1.59	1.53	1.30	1.33	1.28	1.39
Total Arg	1.16	1.16	1.10	1.08	1.11	1.07
Total Trp	0.23	0.21	0.17	0.18	0.17	0.17
Total Gly + Ser	1.61	1.49	1.24	1.44	1.55	1.78
Gly _{equi}	1.36	1.26	1.05	1.24	1.36	1.57

Abbreviations: AME_n, nitrogen-corrected apparent metabolizable energy; CTL, control; dEB, dietary electrolyte balance; LCP, low crude protein; RCP, reduced crude protein.

¹For the mixing of the LCP diets, LCP88 was considered the basal and LCP124 the summit diet and these diets were blended to create the LCP100 and LCP 112 diets.

²The inclusion of cellulose in the RCP diet was formulated for use in a separate experiment as an inert filler, and served no relevant purpose in this study.

³The vitamin premix contained (per kg of diet): vitamin A, 6,350 IU; vitamin D₃, 4,536 ICU; vitamin E, 45 IU; vitamin B₁₂ 0.01 mg; menadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

⁴The mineral premix contained (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

⁵Supplied 0.12 mg of selenium per kg of diet.

⁶IntelliBond C, Micronutrients, Indianapolis, IN.

⁷Optiphos, Huvepharma Inc., Peachtree City, GA.

had higher FI ($P < 0.05$) and FCR ($P < 0.05$) but similar BWG ($P > 0.05$) when compared to birds fed either the CTL or RCP diet. Increasing tGly + Ser in the LCP88 diet linearly lowered FI ($P = 0.007$) and FCR ($P < 0.001$) without affecting BWG ($P > 0.05$).

0 to 39 d Performance Reducing CP (CTL vs. RCP) increased broiler FI ($P = 0.016$) and BWG

($P = 0.002$) and lowered FCR ($P = 0.037$). Broilers fed the LCP88 diet had a lower BWG ($P = 0.002$), higher FCR ($P < 0.001$), and similar FI ($P > 0.05$) when compared to the RCP-fed, but only a higher FCR ($P = 0.005$) and similar ($P > 0.05$) BWG and FI when compared to CTL-fed. Increasing tGly + Ser concentration in the LCP diet linearly reduced FI

Table 7. Live performance of Ross 708 male broilers fed reduced CP diets with moderate or high added Gly levels from 0 to 48 d post-hatch in experiment 1.^{1,2}

Item	CTL	RCP	M Gly	H Gly	SEM	<i>P</i> -values
0 to 14 d						
d 0 BW, kg/bird	0.039	0.039	0.039	0.039	0.0001	0.202
d 14 BW, kg/bird	0.380	0.388	0.393	0.381	0.0050	0.257
BWG, kg/bird	0.341	0.348	0.353	0.341	0.0050	0.279
FI, kg/bird	0.437	0.446	0.445	0.433	0.0050	0.193
FCR ³ , kg:kg	1.283	1.286	1.265	1.284	0.0090	0.342
0 to 28 d						
d 28 BW, kg/bird	1.409 ^a	1.404 ^{a,b}	1.412 ^a	1.364 ^b	0.0113	0.016
BWG, kg/bird	1.369 ^a	1.365 ^{a,b}	1.373 ^a	1.324 ^b	0.0113	0.015
FI, kg/bird	1.880	1.909	1.896	1.867	0.0137	0.177
FCR ³ , kg:kg	1.376 ^b	1.401 ^{a,b}	1.387 ^b	1.419 ^a	0.0077	0.003
0 to 39 d						
d 39 BW, kg/bird	2.573 ^a	2.575 ^a	2.571 ^a	2.476 ^b	0.0201	0.003
BWG, kg/bird	2.533 ^a	2.536 ^a	2.532 ^a	2.437 ^b	0.0200	0.003
FI, kg/bird	3.691 ^{a,b}	3.756 ^a	3.749 ^a	3.650 ^b	0.0236	0.009
FCR ³ , kg:kg	1.471 ^b	1.485 ^b	1.488 ^{a,b}	1.513 ^a	0.0067	0.001
0 to 48 d						
d 48 BW, kg/bird	3.663 ^a	3.669 ^a	3.647 ^a	3.544 ^b	0.0260	0.006
BWG, kg/bird	3.623 ^a	3.630 ^a	3.607 ^a	3.506 ^b	0.0260	0.006
FI, kg/bird	5.563	5.644	5.606	5.518	0.0344	0.074
FCR ³ , kg:kg	1.548 ^b	1.569 ^{a,b}	1.565 ^{a,b}	1.588 ^a	0.0068	0.003

Abbreviations: BWG, body weight gain; CTL, control; FI, feed intake; H, high; M, moderate; RCP, reduced crude protein.

^{a,b}Means within a row that do not share a common superscript were deemed different ($P < 0.05$) by a Tukey's multiple comparison test.

¹Values are least square means of 12 replicate pens.

²The M Gly and H Gly diets had an overall tGly + Ser average of 100 or 112%, respectively, of the CTL diet and 110% and 124%, respectively, of the RCP diet.

³Corrected for mortality.

($P = 0.017$) and FCR ($P = 0.004$) without affecting BWG ($P > 0.05$).

0 to 48 d Performance Cumulatively, broilers fed reduced CP diets had similar ($P > 0.05$) BWG and FCR but did increase FI ($P = 0.022$) when compared to CTL birds. Further CP reductions in the LCP88 diet did not affect FI ($P > 0.05$) but lowered BWG ($P = 0.027$) and increased FCR ($P = 0.004$) compared to the RCP-fed. When compared to the CTL-fed birds, broilers fed the LCP88 diet had higher FCR ($P < 0.001$) but similar ($P > 0.05$) BWG and FI. However, increasing tGly + Ser

linearly improved FCR ($P < 0.001$) and tended to reduce FI ($P = 0.052$). The period and cumulative mortality were unaffected ($P > 0.05$) by treatments (data not shown). Comparing to breeder performance objectives, 0 to 48 d average BW of 3.452 kg and FCR of 1.588 are close to suggested objectives (3.622 kg and 1.692, respectively).

Plasma AA On d 14, increasing tGly + Ser levels in LCP diet linearly increased plasma levels of Cys ($P = 0.047$) and Ser ($P < 0.001$; Table 10). Gly ($P = 0.024$), Trp ($P = 0.009$), and Glu ($P = 0.022$) levels

Table 8. Carcass parts characteristics of Ross 708 male broilers fed reduced CP diets with moderate or high added Gly levels from 0 to 48 d post-hatch in experiment 1.^{1,2}

Item		CTL	RCP	M Gly	H Gly	SEM	<i>P</i> -values
Hot carcass	Weight, kg	2.848 ^a	2.829 ^{a,b}	2.812 ^{ab}	2.742 ^b	0.0244	0.020
	Yield, %	77.46 ^a	77.32 ^{a,b}	77.33 ^{a,b}	76.85 ^b	0.128	0.009
Fat pad	Weight, kg	0.038 ^b	0.042 ^{a,b}	0.046 ^a	0.044 ^{a,b}	0.0016	0.017
	Yield, %	1.04 ^b	1.13 ^{a,b}	1.25 ^a	1.23 ^a	0.044	0.007
Chilled carcass	Weight, kg	2.888 ^a	2.867 ^{a,b}	2.853 ^{a,b}	2.779 ^b	0.0247	0.020
	Yield, %	78.54 ^a	78.33 ^{a,b}	78.45 ^{a,b}	77.91 ^b	0.145	0.019
Breast fillets ³	Weight, kg	0.881	0.885	0.886	0.855	0.0114	0.184
	Yield, %	23.96	24.13	24.33	23.92	0.184	0.371
Tenders ⁴	Weight, kg	0.161 ^a	0.161 ^a	0.157 ^{a,b}	0.150 ^b	0.0020	0.007
	Yield, %	4.38	4.40	4.30	4.33	0.052	0.473
Total breast meat	Weight, kg	1.042	1.046	1.043	1.005	0.0122	0.071
	Yield, %	28.33	28.53	28.65	28.14	0.192	0.268
Wings	Weight, kg	0.285	0.278	0.276	0.276	0.0029	0.134
	Yield, %	7.76	7.61	7.61	7.74	0.055	0.100
Leg quarters	Weight, kg	0.843 ^a	0.828 ^{a,b}	0.823 ^{a,b}	0.798 ^b	0.0083	0.003
	Yield, %	22.95 ^a	22.63 ^{a,b}	22.65 ^{a,b}	22.38 ^b	0.127	0.025

Abbreviations: BWG, body weight gain; CTL, control; FI, feed intake; H, high; M, moderate; RCP, reduced crude protein.

^{a,b}Means within a row that do not share a common superscript were deemed different ($P < 0.05$) by a Tukey's multiple comparison test.

¹Values are least square means of 12 replicate pens.

²The M Gly and H Gly diets had an overall tGly + Ser average of 100 or 112%, respectively, of the CTL diet and 110% and 124%, respectively, of the RCP diet.

³Pectoralis major.

⁴Pectoralis minor.

Table 9. Live performance of Ross 708 male broilers fed reduced CP diets supplemented with Gly from 0 to 48 d post-hatch in experiment 2.¹

Item	Treatments ²							P-values				
	CTL	RCP	LCP88	LCP100	LCP112	LCP124	SEM	CTL vs. RCP	CTL vs. LCP88	RCP vs. LCP88	Glycine	
											Linear	Quadratic
0 to 14 d												
d 0 BW, kg/bird	0.047	0.046	0.046	0.046	0.046	0.046	0.0002	0.574	0.013	0.046	0.185	0.492
d 14 BW, kg/bird	0.443	0.438	0.460	0.447	0.458	0.449	0.0055	0.462	0.033	0.006	0.398	0.771
BWG, kg/bird	0.397	0.392	0.414	0.401	0.412	0.402	0.0048	0.476	0.024	0.004	0.361	0.763
FI, kg/bird	0.488	0.488	0.526	0.506	0.511	0.498	0.0052	0.978	<0.001	<0.001	0.002	0.475
FCR ³ , kg:kg	1.244	1.276	1.278	1.271	1.261	1.252	0.0089	0.005	0.005	0.850	0.027	0.935
0 to 28 d												
d 28 BW, kg/bird	1.475	1.478	1.482	1.460	1.480	1.466	0.0115	0.854	0.683	0.810	0.612	0.695
BWG, kg/bird	1.429	1.431	1.436	1.414	1.434	1.420	0.0115	0.859	0.648	0.768	0.601	0.699
FI, kg/bird	1.965	1.976	2.015	1.969	1.989	1.951	0.0136	0.489	0.008	0.039	0.007	0.798
FCR ³ , kg:kg	1.378	1.384	1.408	1.394	1.391	1.378	0.0041	0.228	<0.001	<0.001	<0.001	0.913
0 to 39 d												
d 39 BW, kg/bird	2.633	2.709	2.626	2.598	2.614	2.611	0.0184	0.002	0.761	0.001	0.734	0.495
BWG, kg/bird	2.587	2.662	2.580	2.552	2.568	2.565	0.0184	0.002	0.792	0.002	0.721	0.487
FI, kg/bird	3.828	3.888	3.872	3.824	3.848	3.792	0.0197	0.016	0.096	0.554	0.017	0.843
FCR ³ , kg:kg	1.482	1.469	1.501	1.500	1.499	1.480	0.0050	0.037	0.005	<0.001	0.004	0.071
0 to 48 d												
d 48 BW, kg/bird	3.472	3.516	3.437	3.420	3.477	3.391	0.0257	0.168	0.316	0.026	0.483	0.181
BWG, kg/bird	3.425	3.469	3.391	3.374	3.431	3.345	0.0257	0.169	0.321	0.027	0.479	0.179
FI, kg/bird	5.382	5.470	5.438	5.373	5.454	5.320	0.0305	0.022	0.180	0.423	0.052	0.262
FCR ³ , kg:kg	1.574	1.579	1.601	1.598	1.592	1.584	0.0056	0.469	<0.001	0.004	0.033	0.682

Abbreviations: BWG, body weight gain; CTL, control; FI, feed intake; LCP, low crude protein; RCP, reduced crude protein.

¹Values are least square means of 12 replicate pens for the control and RCP diets and 9 replicate pens for the LCP diets.

²The LCP88, LCP100, LCP112, and LCP124 had an 88, 100, 112, and 124% increase in tGly + Ser, respectively, compared to the RCP diet.

³Corrected for mortality.

responded quadratically. Plasma levels of Met, Lys, Thr, Val, Ile, Leu, Arg, Asp, Ala, Pro, Phe, Tyr, and His were not affected ($P > 0.05$) by increasing dietary tGly + Ser levels.

On d 48, Gly ($P = 0.028$) and Ser ($P = 0.035$) plasma levels increased quadratically to increasing tGly + Ser levels (Table 11). Met ($P = 0.041$) and Pro ($P = 0.046$) levels also had quadratic responses ($P < 0.05$). Plasma levels of Cys, Lys, Thr, Val, Ile, Leu, Arg, Gly, Asp, Glu, Ala, Trp, Phe, Tyr, and His were not affected ($P > 0.05$).

Processing Absolute and relative fat pad weights were similar ($P > 0.05$) for the CTL and RCP groups, but were heavier ($P < 0.05$) for the LCP88 group (Table 12). Increasing tGly + Ser in the LCP diet linearly reduced absolute ($P < 0.001$) and relative ($P < 0.001$) fat pad weights, but did not affect ($P > 0.05$) hot and chilled carcass weight and yield. Hot carcass weight ($P = 0.009$) of the RCP group and yield ($P = 0.027$) of the CTL group were greater than these same values for the LCP88 group, with no other treatment effects ($P >$

Table 10. D 14 plasma amino acids ($\mu\text{mol/L}$) of Ross 708 male broilers fed reduced CP diets supplemented with Gly from 0 to 14 d post-hatch in experiment 2.¹

Item	Treatments ²					P-values		
	LCP88	LCP100	LCP112	LCP124	SEM	Linear	Quadratic	
Met	122	131	141	144	11.3	0.143	0.765	
Cys	36	37	40	43	2.8	0.047	0.816	
Lys	196	224	200	197	24.9	0.857	0.533	
Thr	1,288	1,115	1,065	1,225	106.8	0.618	0.130	
Val	196	201	201	210	18.1	0.618	0.920	
Ile	90	96	95	99	9.4	0.516	0.906	
Leu	181	186	173	187	15.5	0.930	0.769	
Arg	1,493	633	1,417	886	213.2	0.286	0.446	
Gly	389	644	956	1,450	52.4	<0.001	0.024	
Ser	327	383	466	519	32.8	<0.001	0.954	
Asp	25	38	28	30	3.0	0.267	0.721	
Glu	206	141	132	135	14.0	0.001	0.022	
Ala	944	755	884	876	89.9	0.857	0.323	
Pro	571	524	607	568	50.6	0.744	0.938	
Trp	1,255	1,393	1,815	1,145	145.2	0.888	0.009	
Phe	218	190	226	221	21.4	0.647	0.601	
Tyr	230	189	207	208	21.0	0.601	0.327	
His	32	38	34	38	3.7	0.408	0.710	

Abbreviation: LCP, low crude protein.

¹Values are least square means of 9 replicate pens.

²The LCP88, LCP100, LCP112, and LCP124 had an 88, 100, 112, and 124% increase in tGly + Ser, respectively, compared to the RCP diet.

Table 11. D 48 plasma amino acids ($\mu\text{mol/L}$) of Ross 708 male broilers fed reduced CP diets supplemented with Gly from 0 to 48 d post-hatch in experiment 2.¹

Item	Treatments ²				SEM	P-values	
	LCP88	LCP100	LCP112	LCP124		Linear	Quadratic
Met	152	143	119	143	7.8	0.144	0.041
Cys	34	29	26	29	2.0	0.063	0.068
Lys	142	107	113	117	14.1	0.284	0.181
Thr	570	529	504	593	38.0	0.785	0.096
Val	157	145	138	155	10.6	0.779	0.185
Ile	78	77	71	81	6.5	0.905	0.377
Leu	165	166	156	165	11.1	0.865	0.723
Arg	352	366	278	325	21.2	0.077	0.421
Gly	442	685	692	1,150	46.6	< 0.001	0.028
Ser	474	460	455	604	36.9	0.026	0.035
Asp	32	29	30	32	2.5	0.974	0.315
Glu	165	151	138	143	8.7	0.051	0.304
Ala	800	779	634	750	49.2	0.191	0.175
Pro	430	418	368	450	22.8	0.941	0.046
Trp	1,183	726	430	569	290.5	0.092	0.292
Phe	133	129	117	121	8.6	0.228	0.680
Tyr	260	239	202	231	14.4	0.063	0.086
His	27	26	23	24	1.7	0.114	0.332

Abbreviation: LCP, low crude protein.

¹Values are least square means of 9 replicate pens.

²The LCP88, LCP100, LCP112, and LCP124 had an 88, 100, 112, and 124% increase in tGly + Ser, respectively, compared to the RCP diet.

0.05) on hot carcass measurements. Chilled carcass weight ($P < 0.05$) was greater for the RCP group when compared to the CTL or LCP88 group, with no other treatment effects ($P > 0.05$) on chilled carcass weight or yield observed.

For deboned parts, comparing the CTL and RCP groups, breast fillet and total breast meat weight and yield were greater ($P < 0.05$) for the RCP group, whereas wing yield ($P < 0.001$), but not weight ($P > 0.05$), was greater for the CTL group. Leg quarter and tender weight and yield were similar ($P > 0.05$) between the CTL and RCP groups. When comparing the CTL and LCP88 groups, tender weight

($P = 0.003$) and yield ($P < 0.001$), total breast meat ($P = 0.020$) and wing ($P = 0.049$) yield, but not ($P > 0.05$) weight of these parts, were greater for the CTL group. Leg quarter and breast fillet weight and yield were similar ($P > 0.05$) between the CTL vs. LCP88 groups. For the RCP vs. LCP88 comparison, breast fillet, tender, and total breast meat weight and yield were reduced ($P < 0.05$) in the LCP group. Wing weight and yield were not impacted ($P > 0.05$). Leg quarter yield ($P = 0.006$), but not weight ($P > 0.05$), was greater for the LCP88 group compared to the RCP group. Increasing tGly + Ser in the LCP88 diet did not affect ($P > 0.05$) deboned parts weights

Table 12. Carcass parts characteristics of Ross 708 male broilers fed reduced CP diets supplemented with Gly from 0 to 48 d post-hatch in experiment 2.^{1,2}

Item		Treatments						SEM	P-values				
		CTL	RCP	LCP88	LCP100	LCP112	LCP124		CTL vs. RCP	CTL vs. LCP88	RCP vs. LCP88	Glycine	
											Linear	Quadratic	
Hot carcass	Weight, kg	2.629	2.700	2.585	2.593	2.612	2.564	0.032	0.076	0.301	0.009	0.759	0.384
	Yield, %	76.86	76.77	76.29	76.30	75.96	76.43	0.188	0.692	0.027	0.061	0.933	0.235
Fat pad	Weight, kg	0.040	0.043	0.051	0.049	0.046	0.046	0.0015	0.105	<0.001	<0.001	0.003	0.538
	Yield, %	1.16	1.22	1.52	1.45	1.34	1.36	0.038	0.281	<0.001	<0.001	0.001	0.243
Chilled carcass	Weight, kg	2.678	2.758	2.639	2.637	2.667	2.617	0.0326	0.050	0.364	0.008	0.811	0.460
	Yield, %	78.29	78.41	77.88	77.61	77.60	78.01	0.233	0.680	0.195	0.096	0.723	0.150
Breast fillets ³	Weight, kg	0.798	0.844	0.774	0.779	0.774	0.762	0.0145	0.012	0.217	<0.001	0.537	0.561
	Yield, %	23.30	23.97	22.77	22.87	22.46	22.69	0.237	0.025	0.101	<0.001	0.548	0.782
Tenders ⁴	Weight, kg	0.150	0.152	0.140	0.137	0.136	0.138	0.0022	0.442	0.003	<0.001	0.372	0.363
	Yield, %	4.37	4.31	4.14	4.11	3.97	4.10	0.041	0.249	<0.001	0.003	0.157	0.054
Total breast meat	Weight, kg	0.947	0.996	0.914	0.916	0.910	0.900	0.0158	0.015	0.117	<0.001	0.489	0.685
	Yield, %	27.67	28.28	26.91	26.92	26.43	26.79	0.238	0.040	0.020	<0.001	0.429	0.462
Wings	Weight, kg	0.274	0.273	0.266	0.266	0.27	0.266	0.0033	0.871	0.098	0.131	0.813	0.578
	Yield, %	8.02	7.77	7.87	7.84	7.86	7.94	0.055	<0.001	0.049	0.206	0.339	0.340
Leg quarters	Weight, kg	0.773	0.786	0.775	0.766	0.788	0.776	0.0097	0.288	0.882	0.408	0.575	0.902
	Yield, %	22.62	22.36	22.92	22.57	22.93	23.14	0.144	0.148	0.128	0.006	0.116	0.063

Abbreviations: BWG, body weight gain; CTL, control; FI, feed intake; LCP, low crude protein; RCP, reduced crude protein.

¹Values are least square means of 12 replicate pens for the control and RCP diets and 9 replicate pens for the LCP diets.

²The LCP88, LCP100, LCP112, and LCP124 had an 88, 100, 112, and 124% increase in tGly + Ser, respectively, compared to the RCP diet.

³Pectoralis major.

⁴Pectoralis minor.

or yields, however, there was a tendency ($P = 0.054$) for increased tender yield with increasing tGly + Ser.

DISCUSSION

The many benefits of implementing reduced CP diets in broiler production have been reviewed extensively (Powers and Angel, 2008; Greenhalgh et al., 2020; Capelaere et al., 2021). The ongoing challenge in successfully reducing dietary CP has been to determine the nutrients that limit performance once CP reductions meet certain thresholds (as reviewed by Aftab et al., 2006). One nutrient of potential importance in this regard is Gly, a conditionally-essential AA, which has been reported to improve young broiler growth when added to reduced CP diets, especially when all vegetable based diets are used. However, there is a limited amount of published data to determine if the beneficial effects of increasing dietary tGly + Ser in all diet phases will extend beyond early growth to improve overall performance and meat yield at market ages. Although there are currently no published Gly recommendations from primary breeders (Cobb-Vantress, 2018; Aviagen, 2019), Waguespack et al. (2009b) determined that Ross 708 broilers had a requirement of 2.10% tGly + Ser from 0 to 18 d. Others have found optimum tGly + Ser ranging from 1.8 to 2.3% from 0 to 35 d (Corzo et al., 2004; Waldroup et al., 2005; Dean et al., 2006; Ospina-Rojas et al., 2013a,b). Depending on the degree of CP reduction and ingredients used (e.g., all vegetable vs. animal protein), incorporating feed-grade AA without setting a minimum for CP can often result in tGly + Ser levels below these recommendations.

In the current research, the CTL diet for both experiments used only feed-grade L-Met, L-Lys, and L-Thr to mimic a commercially relevant diet. In addition to these AA, the RCP diet included L-Val and L-Ile and L-Arg (experiment 2 from 28 to 48 d). Although L-Val, L-Ile, and L-Arg are not all routinely used into formulation, they are becoming more widely available and economical to implement. The LCP diets were formulated with greater feed-grade AA inclusion levels at each feed phase as well the addition of L-Trp (39 to 48 d) to further reduce CP and arrive at tGly + Ser lower than previously mentioned recommended levels. The CTL diet in experiment 1 vs. 2 had a higher average analyzed CP and tGly + Ser levels by 1.3 and 0.2% units, respectively, whereas the RCP diet in experiment 1 vs. 2 had comparable levels by 0.2 and 0.0% units, respectively. The M Gly and H Gly diets had approximately a 2.2% unit higher calculated CP content than the LCP diets with the tGly + Ser level of the M Gly diet between the levels of the LCP100 and LCP112 diets and the H Gly and LCP124 diets having similar (0.01% unit difference) tGly + Ser levels.

In experiment 1, reducing dietary CP by 1.68% units and maintaining essential AA levels resulted in equivalent broiler growth and deboned part yields from 0 to 48 d. Because there was not an apparent limitation in broiler performance when feeding the RCP diet, it is not

surprising that increasing Gly in the RCP diet to 100 or 112% of the tGly + Ser levels of the CTL diet (starter phase, 2.17%) did not elicit a response in performance. Increasing tGly + Ser levels in a CTL or non-limiting nutrient diet has been shown to result in no effect (Dean et al., 2006) or inconsistent responses (Waguespack et al., 2009a). Interestingly, exceeding the tGly + Ser levels of the CTL diet in the H Gly diet was detrimental to broiler BWG and FCR. This may be due to an increased energy, Gln, and Asp demands for uric acid synthesis to excrete excess Gly (Selle et al., 2021; van Milgen, 2021).

In experiment 2, broilers that were offered diets with a marginal CP reduction (0.64% units; RCP diet) from 0 to 48 d also had equal growth performance to the CTL diet, which is in agreement with experiment 1. Chicks fed a lower CP diet (LCP88) during the starter phase had greater feed consumption (38 g) and BWG (20 g) than chicks fed either the CTL or RCP diet. The increased FI of the LCP88 fed chicks may have resulted from chicks eating to satisfy a Gly need, and indeed, graded increases in tGly + Ser in the LCP diets resulted in a linear reduction in chick FI and FCR. Furthermore, the increased FI of the LCP88 chicks likely explains the heavier BWG than the CTL or RCP groups. Chicks fed a 20% CP diet in experiment 2 needed a tGly + Ser content higher than 1.80% (LCP88) and responded linearly up to 2.55% tGly + Ser from 0 to 14 d. This is in contrast to results from experiment 1 where increasing tGly + Ser levels above 1.95% in a 22% CP diet did not benefit performance.

During the 0 to 39 d period, broilers fed the RCP diet had a higher BWG and FI with a lower FCR than the CTL-fed broilers, although these improvements in BWG and FCR were not captured in other periods or overall growth. These results are likely due to broilers eating to satisfy a certain nutrient need like previously recorded for the LCP-fed chicks from 0 to 14 d and such nutrients may include the lower (7.4-8.3%) analyzed tGly+Ser, Trp, or Leu from 28 to 39 d. From 0 to 48 d, broilers fed the LCP88 diet (2.38% unit average analyzed CP reduction) had the highest FCR and linear, but not quadratic, improvements in FCR were observed when increasing dietary tGly + Ser. Similarly, other studies have shown that the addition of Gly had the most impact on improving FCR (Waguespack et al., 2009b; Berres et al., 2010; Kriseldi et al., 2017), possibly by improving nutrient utilization and protein synthesis via enterocyte development or mucin secretion (Powell et al., 2009, 2011; Wang et al., 2020). A lack of a quadratic response may indicate that optimum tGly + Ser levels were not reached even at the highest level of added Gly from 0 to 48 d.

A meta-analysis conducted by Siegert et al. (2015) estimated the Gly_{equi} requirements of broilers at 95% of the maximum response to be 1.61% and 1.58% Gly_{equi} for ADG and G:F, respectively. These authors formulated Gly and Ser on Gly_{equi} basis as it accounts for the conversion of Ser to Gly on a molar basis at 71.43% (Dean et al., 2006). The authors herein have reported the Gly_{equi} in Tables 1-6 as a reference. However, a re-evaluation of this

Gly_{equi} conversion may be warranted to account for the balance of 1-carbon units as 2 of the 5 carbon atoms needed to synthesize uric acid originate from 1-carbon units (van Milgen, 2021). Van Milgen (2021) suggested that the value of Ser relative to Gly ranges from 71.43% to 142.9% because one mol of Ser can synthesize two 1-carbon units, whereas Gly can synthesize one. Others have reported Gly and Ser concentrations as tGly + Ser or as a ratio of tGly + Ser to digestible Lys. Further work is needed to determine the best approach to formulate Gly and Ser.

Positive responses to supplementing Gly depend not only on the Gly and Ser levels but also the levels of their precursors and metabolites such as Met, Cys, Thr, Arg, Gln, Asp, CP, choline, guanidino acetic acid, creatine, and betaine (Siegert et al., 2015; Belloir et al., 2017; Siegert and Rodehutschord, 2019). The H Gly diet in experiment 1, which resulted in the lowest performance among the RCP diets, and the LCP124 diet in experiment 2, which resulted in the best performance among the LCP diets, had similar tGly + Ser concentrations and comparable choline, Thr, and Arg levels, but differed in CP by approximately 11% (CP difference between the H Gly and LCP). As CP is reduced, feed-grade Met is supplemented to maintain dTSAA levels; however, Cys may then become potentially limiting in corn-soybean meal based diets. Indeed, the dietary Met to Cys ratio has been suggested to impact Gly requirements, and this ratio differed between diets fed in the 2 current experiments (Powell et al., 2011; Siegert et al., 2015). The Met to Cys ratio ranged from 69:31 to 68:32 and 74:26 to 73:27 across feeding phases in experiments 1 and 2, respectively. The conversion of Met to Cys requires the transformation of Met to L-homocysteine and one molecule of Ser for the synthesis of L,L-cystathionine. The meta-analysis by Siegert et al. (2015) determined an optimal Met to Cys ratio of 66:34 and a Cys concentration of 0.30%; at these levels the Gly + Ser requirement was lowest. These authors confirmed that increasing Met to Cys ratio increased the requirement of Gly + Ser, whereas increasing Cys concentration lowered the need for maintaining Gly + Ser (Siegert et al., 2015). Indeed, increasing tGly + Ser levels with Cys levels at or above estimated Cys requirements did not affect performance (Powell et al., 2011). Every molecule of Met converted to Cys requires 2 units of Gly_{equi} and ammonia. Ammonia can then be used for the synthesis of nonessential AA or detoxified to uric acid, thus requiring more Gly (Karasawa, 1989; Siegert and Rodehutschord, 2019; Hofmann et al., 2020).

In addition to differences in dietary CP and AA profile between experiments 1 and 2, rearing conditions may also have influenced broiler responses to dietary Gly. In experiment 1, birds were reared on fresh, unused pine shavings, whereas in experiment 2 birds had a bedding of de-caked litter that was top-dressed with fresh pine shavings. Kidd et al. (2003) and Corzo et al. (2007) reported that broilers reared on used vs. new litter had a 2 to 10% higher dietary Thr need. These authors suggested that higher Thr needs are related to increased

intestinal mucosal maintenance as a consequence of greater microbial exposure from used litter. The intestinal mucosa has an integral role as the first line of defense to pathogens entering the gastrointestinal tract, and its composition is rich in Thr, Ser, Pro and also relatively moderate amounts of Gly (Mantle and Allen, 1981). A pathogen exposure such as *Eimeria* spp. can increase specific AA needs in order to repair intestinal damage and support immune responses (as reviewed by Lee and Rochell, 2022) Further research is needed to quantify broiler tGly + Ser needs with different rearing environments and associated pathogen presence.

Plasma AA in LCP-fed birds were analyzed at d 14 and 48 in experiment 2. As dietary levels of tGly + Ser increased, plasma Gly and Ser concentrations also increased. Cys levels at d 14, but not at d 48, were linearly increased as tGly + Ser increased, possibly supporting elevated Gly needs to support the conversion of Met to Cys for young broilers. On d 48, a quadratic Met response with increasing tGly + Ser could have been due to a lower need for Met conversion to Cys as dietary Gly availability increased. Plasma Glu and Pro increased while Trp decreased in a quadratic fashion with graded increases in supplemental Gly. These AA may have accumulated or dissipated in the plasma because they were in excess to another nutrient or became limiting, respectively, for protein synthesis (Johnson and Anderson, 1982; Erwan et al., 2020). Hofmann et al. (2019) found Glu, Pro, Asn, and Gln plasma concentrations increased with increased tGly + Ser levels, but several other plasma AA concentrations interacted with Gly and CP levels. Although not evaluated in this study, metabolic intermediates such as the ones studied by Elahi et al. (2020) and Hofmann et al. (2019) could further our understanding of the upregulation or downregulation of certain pathways in response to Gly, Ser, Met, and Cys relationship and this should be considered in future studies. A limitation of the current study is that we were not able to collect blood from birds in the control groups (CTL and RCP) for comparison due to their use in another concurrent experiment. Nonetheless, the changes in plasma AA in response to graded increases in dietary tGly + Ser levels still provide an indication of the limited or excess AA available to peripheral tissues.

It has been commonly observed that broilers fed lower CP diets will increase abdominal fat pad deposition (Moran and Stilborn, 1996; Namroud et al., 2008; Krijseldi et al., 2017). This appears to be due to an increase in dietary calorie to protein ratio as well as birds having greater heat increment when surplus AA are deaminated and transaminated in their conversion to other metabolites and uric acid (Bartov et al., 1974; Namroud et al., 2008). In experiment 1, feeding Gly in excess of the bird's apparent need may have overwhelmed the bird's capacity to mobilize Gly via uric acid and caused this excess Gly or another limiting nutrient to affect lipid metabolism. In experiment 2, birds fed the LCP88 diet had greater absolute and relative fat pad weights than the CTL or RCP group, and increasing the concentration of tGly + Ser linearly reduced abdominal fat

deposition, though the lowest relative fat pad weight of all the LCP groups was still 16% higher than that of the CTL group. These results are in agreement with [Elahi et al. \(2020\)](#) who showed that supplementing Gly in reduced CP diets can lower fat pad deposition, but not to the level of control-fed broilers. Increasing dietary Gly or betaine (trimethylglycine) has been demonstrated to stimulate lipid oxidation and reduce fat deposition in poultry, rats, and swine ([El Hafidi et al., 2004](#); [Fouad and El-Senousey, 2014](#); [Zhong et al., 2021](#)); however, the role of Gly on broiler lipid metabolism needs further elucidation.

Reducing CP did not negatively affect deboned parts weights or yields in either experiment, excluding wing yield (experiment 2). In fact, broilers fed the RCP diet (experiment 2) had greater breast meat accretion, as previously discussed by [Lee et al. \(2020\)](#). In experiment 1, feeding the highest Gly diet (H Gly) negatively impacted leg quarter and tender yields. Excess Gly that was excreted could have increased energy demand as well as certain AA (i.e., Gln and Asp) that would have otherwise been used to promote tissue deposition. In experiment 2, the low CP diet (LCP88) was detrimental to pectoralis minor (tender) weight and yield, and increasing tGly + Ser tended to restore pectoralis minor growth. [Hilliari et al. \(2019, 2020\)](#) found that the addition of feed-grade Gly in a reduced CP diet (approximately 3% units) reduced breast meat yield compared with birds fed no added Gly (0.86% and 1.30% dig Gly + Ser, respectively), the cause of which is unclear. It was previously hypothesized that an increase in dietary Gly may promote creatine biosynthesis to enhance breast and dark muscle development ([Hegsted, 1944](#); [Fisher et al., 1955](#); [Ngo et al., 1977](#); [Ospina-Rojas et al., 2013a](#)). [Ospina-Rojas et al. \(2013a\)](#) found that increased dietary tGly + Ser linearly improved d 21 breast muscle weight and creatine concentrations. At d 48 in the current study, breast muscle weight and yield were not affected by dietary tGly + Ser levels. [Corzo et al. \(2021\)](#) reported that increasing dietary Arg levels yielded greater thigh weight and yields at d 42, possibly due the role of Arg in creatine biosynthesis. Creatine is synthesized from 3 AA: Gly, Arg, and Met. Broilers in experiment 2 did not have greater leg quarter weight or yield when fed increased tGly + Ser levels, but results may have differed if thighs and drums were evaluated separately ([Al-daraji and Salih, 2012](#); [Corzo et al., 2021](#)). Furthermore, [Ngo et al. \(1977\)](#) carefully noted that creatine content in muscle increased with age at a diminishing rate to a point of maximum creatine synthesis. Therefore, the relationship between increased creatine levels and enhanced muscle protein synthesis up to market weight age as stimulated by precursor AA warrants further study.

In conclusion, these data indicate that moderate reductions in CP (1.68% units) from breeder recommendations can be achieved with the use of L-Met, L-Lys, L-Thr, L-Val, and L-Ile without concern for Gly limitation when tGly + Ser meet or exceed 1.95% (starter phase).

However, further CP reductions (2.38% units) achieved with incorporation of additional limiting essential AA, such as L-Arg and L-Trp in this case, may require that tGly + Ser be maintained with added Gly. Further, this research supports previous studies to suggest that tGly + Ser needs likely depend on the concentrations and balance of other nutrients like Met, Cys, and their ratio. Greater CP reductions (approximately more than 3% units from breeder recommendations) may cause nutrients other than Gly to become limiting ([Hofmann et al., 2019](#); [Hilliari et al., 2020](#); [Wang et al., 2020](#)). Future research should investigate the interaction of Gly + Ser and other nutrients as well as determine Gly needs during different environmental conditions and pathogenic stress loads.

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DISCLOSURES

There are no conflicts of interest to declare.

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