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Low crude protein, amino acid-supplemented diets, and the glycine requirement in low crude protein diets for broilers

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LOW CRUDE PROTEIN, AMINO ACID-SUPPLEMENTED DIETS, AND THE GLYCINE REQUIREMENT IN LOW CRUDE PROTEIN DIETS FOR BROILERS

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ABSTRACT

The purpose of this research was to determine the optimal level of crystalline amino acid (AA) supplementation that supports maximum growth performance in broilers, and to determine the Gly requirement in broilers. Treatments were replicated with a minimum of 7 pens with 6 broilers per pen. Experiments (Exp.) were conducted from 0- to 18- days (d) post-hatching in brooder batteries. Three Exp. were conducted to determine the maximum level of L-Lys•HCl that could be supplemented to corn-soybean meal (C-SBM) diets without negatively affecting growth performance of broilers. The results of these Exp. indicate that broilers can achieve maximum growth performance when fed a C-SBM diet supplemented with 0.25% L-Lys•HCl. Two Exp. were conducted to determine the fifth, sixth, and seventh limiting AA in a low crude protein (CP), C-SBM diet in broilers. The results of these Exp. indicate that Arg and Val may be equally limiting followed by Ile in a C-SBM diet supplemented with DL-Met, L-Lys•HCl, L-Thr, and Gly. Three Exp. were conducted to determine the Gly requirement of broiler chicks fed a low CP, AA-supplemented diet. The results of this research indicate that 2.078% total Gly + Ser is required for broilers fed a C-SBM diet supplemented with 0.25% L-Lys•HCl.

CHAPTER 1

INTRODUCTION

Formulating broiler diets to maximize growth performance, decrease excess nitrogen in the excreta, and to maximize economic returns are primary considerations of the broiler industry. The most cost effective, and perhaps the only way, to minimize nitrogen in the excreta is to formulate diets with reduced levels of CP and with inclusion of the commercially available AA (DL-Met, L-Lys, L-Thr, and L-Trp). However, reducing CP in broiler diets by inclusion of supplemental crystalline AA has not been successful until recently (Dean et al., 2006). Previously, decreasing CP content by more than 3% resulted in decreased weight gain and feed efficiency and increased abdominal fat. Dean et al. (2006), however, reported that maximum growth performance could be obtained in low CP, AA-supplemented diets if those diets were supplemented with crystalline Gly. The diet used by Dean et al. (2006) contained many supplemental AA (L-Lys•HCl, L-Arg•HCl, DL-Met, L-Thr, L-Val, L-Ile, L-Phe, L-His, Gly, L-Trp, and L-Leu) and contained 16% CP, which is not a practical commercial diet. Therefore, the objective of this research was to determine the optimal level of crystalline AA supplementation that could be incorporated into a C-SBM diet with no negative effects on growth performance, and then to determine the Gly requirement of broilers fed this diet.

CHAPTER 2

REVIEW OF LITERATURE

INTRODUCTION

Environmental issues with nitrogen runoff from excessive protein intake of confined livestock have caused an increase in research to decrease nitrogen loss to the environment (Morse, 1995). Crystalline AA supplementation to diets fed to broilers will reduce dietary CP levels; therefore, reducing nitrogen excretion (Aletor et al., 2000). However, researchers have reported negative growth performance effects such as reduced feed efficiency and increased abdominal fat when broilers were fed low CP, AA-supplemented diets (Pinchasov et al., 1990; Bregendahl et al., 2002; Fancher and Jensen, 1989b). Previous attempts to correct the negative growth effects of broilers fed low CP diets have been to modify the dietary electrolyte balance (DEB) and supplement the diet with non-specific nitrogen (NSN) and (or) AA (Aftab et al., 2006).

DIETARY ELECTROLYTE BALANCE

Attempts to modify the DEB were based on the reduction in the dietary potassium content when SBM in the AA-supplemented diets was decreased and the chloride content of the diets increased due to the increase in supplemental L-Lys•HCl. However, when low CP diets were supplemented with potassium sulfate to increase the potassium level, the low CP diet still failed to result in growth performance equal to standard diets (Si et al., 2004). Fancher and Jensen (1989b) reported that the addition of potassium carbonate to 19 and 16% CP diets for growing (d 21 to 42) broilers failed to improve broiler weight gain or feed efficiency. In 1- to 3-wk old broilers, Han et al.

(1992) reported that a 19% CP diet supplemented with potassium carbonate had no effect on growth performance.

NON-SPECIFIC NITROGEN

Attempts to modify the NSN additions in the diets were based on the assumption that in high or standard CP diets, birds can synthesize non-essential amino acids (NEAA) from excess essential amino acids (EAA). The thought was that low CP diets would have decreased excess EAA and therefore would negatively affect NEAA synthesis. However, Bregendahl et al. (2002) conducted an Exp. with crystalline NEAA additions and triammonium citrate as a source of NSN, but they were unsuccessful in resulting in maximum growth performance of broilers. Fancher and Jensen (1989a,b) reported that the addition of 5% L-Glu to a 19% CP diet had no effect on 21- to 42-d old male or female broiler weight gain or feed efficiency. Pinchasov et al. (1990) reported that the addition of 3, 6, or 9% L-Glu to a low CP diet failed to improve growth performance of broilers chicks. Supplementation of 1% L-Glu to 17, 15 or 13% CP diets failed to improve 28- to 42-d old broiler performance to the level of broilers fed the 19% CP without L-Glu supplementation (Kerr and Kidd, 1999). Han et al. (1992) reported that the addition of 4.6% L-Glu to a 19% CP diet had no effect on broiler growth performance; however, when at least 2.3% L-Glu was supplemented in combination with crystalline DL-Met, L-Lys, L-Arg, L-Val, and L-Thr, broiler growth performance was improved to the level of broilers fed the control 23% CP diet.

ESSENTIAL AND NON-ESSENTIAL AMINO ACID RATIO

Bedford and Summers (1985) reported for broiler chicks to achieve optimal growth performance a 55:45 ratio of EAA:NEAA should be provided in the diet;

therefore, feeding a 14% CP diet with a ratio of less than 55:45 EAA:NEAA would result in decreased growth performance of broilers.

ADDITION OF ESSENTIAL AND NON-ESSENTIAL CRYSTALLINE AMINO ACIDS

Neither the DEB nor the NSN modifications have been successful in resolving the negative growth performance effects of low CP, AA-supplemented diets fed to broilers (Aftab et al., 2006). Other research has been to evaluate effects of EAA and NEAA supplementation in low CP diets for broilers.

Fancher and Jensen (1989a) reported that supplementing 7.5 or 15% in excess of the NRC requirement with crystalline L-Lys and DL-Met or L-Arg, L-Thr, L-Ile, and L-Trp or all 6 AA to a low CP diet failed to improve body weights of female broilers. Fancher and Jensen (1989b) reported that supplementing 10% in excess of the NRC requirement with crystalline L-Arg, L-Thr, L-Ile, and L-Trp to a low CP diet had no effect on body weight and decreased feed intake; however, the male broilers had increased feed efficiency compared with male broilers fed the 16% CP diet without the addition of these four crystalline AA. Bregendahl et al. (2002) reported that supplementing crystalline EAA to exceed 15, 30, and 45% of the NRC requirements for broilers chicks had no beneficial effect on feed intake or body weight gain. Pinchasov et al. (1990) reported that decreasing the ratio of EAA to 93.5 or 87.5% of NRC requirements as the CP level was reduced did not improve growth performance of broiler chicks.

Bregendahl et al. (2002) reported that the addition of 1% L-Gln or L-Asn to a low CP diet (approximately 19% CP) fed to broilers chicks failed to improve growth performance. When broilers were fed the low CP diets supplemented with L-Glu and EAA such as L-Arg, L-Thr, L-Trp, L-Ile, and L-Val, body weight gain and feed conversion ratio (FCR) were improved, but not to the level of broilers fed the 19% CP diet (Kerr and Kidd, 1999). Leclercq et al. (1994) fed 30- to 44-d old broilers low CP diets supplemented with EAA, L-Glu, and L-Asp and reported that broilers had increased feed intake and FCR compared with broilers fed the 19% CP diet.

Han et al. (1992) reported that 1- to 3-wk old broilers had similar weight gain and feed efficiency when fed a 19% CP diet supplemented with crystalline DL-Met, L-Lys, L-Arg, L-Val, and L-Thr compared with broilers fed the 23% CP diet. Parr and Summers (1991) reported that broiler chicks fed a low CP diet with the addition of Gly had improved feed conversion. Corzo et al. (2005) reported that broilers fed an 18% CP diet with supplemental Gly or Leu had FCR equal to that of broilers fed the control diet. Dean et al. (2006) also reported that broilers fed low CP, AA-supplemented diets had growth performance equal to broilers fed conventional C-SBM diets as long as crystalline Gly was supplemented.

CHAPTER 3

EFFECT OF INCREMENTAL LEVELS OF L-LYSINE•HCL AND DETERMINATION OF THE LIMITING AMINO ACIDS IN LOW CRUDE PROTEIN CORN-SOYBEAN MEAL DIETS FOR BROILER CHICKS

INTRODUCTION

Researchers have shown that feeding low CP diets to broilers will decrease the nitrogen in the excreta; therefore, reducing the nitrogen loss to the environment (Aletor et al. 2000). Researchers also have shown decreased growth performance of broilers fed low CP, AA-supplemented diets (Fancher and Jensen, 1989a,b and Pinchasov et al., 1990).

Attempts to improve broiler growth performance when feeding low CP diets include modification in the DEB or more specifically addition of potassium carbonate to the diet (Fancher and Jensen, 1989a,b; Han et al., 1992; Si et al., 2004), addition of NSN as triammonium citrate or L-Glu (Fancher and Jensen, 1989a,b; Pinchasov et al., 1990; Bregendahl et al., 2002), addition of EAA to meet or exceed the NRC requirements (Fancher and Jensen, 1989a,b; Bregendahl et al., 2002; Pinchasov et al., 1990), or addition of crystalline NEAA (Bregendahl et al., 2002). Neither the DEB nor the NSN modifications have been successful in resolving the negative growth performance effects of low CP, AA-supplemented diets fed to broilers (Aftab et al., 2006). However, EAA and NEAA supplementation in low CP diets have been shown to be relatively successful. Corzo et al. (2005) reported that broilers fed an 18% CP diet with supplemental Gly or L-Leu had FCR equal to that of broilers fed a control 22% CP diet. Dean et al. (2006) also reported that broilers fed low CP, AA-supplemented diets had growth performance equal to broilers fed conventional C-SBM diets as long as crystalline Gly was supplemented. However, the 16% CP diet used by Dean et al. (2006) was not a practical commercial diet because it contained additional crystalline AA other than the commercially available AA. The objective of this research was to determine the level of the commercially available AA (L-Lys•HCl, L-Thr, and DL-Met) along with Gly that could be added to C-SBM diets that would result in no detrimental effects on growth performance.

MATERIALS AND METHODS

Five Exp. were conducted with male Ross × Ross 708 broilers. The Ross x Ross 708 is a slow growing, high breast yield broiler mainly used for deboning and cut-ups (Aviagen, 2007). On d 0 post-hatching, broilers were weighed, wing banded, and randomly allotted to treatments. Broilers were housed in temperature-controlled Petersime starter batteries with continuous fluorescent lighting. Each Exp. was conducted for 18 d. Feed in mash form and water were available on an ad libitum basis. Chicks and feeders were weighed on d 0 and 18 for the determination of growth performance. All experimental animal use was in compliance with the LSU Agricultural Center Animal Care and Use Committee.

Diets were C-SBM based and formulated to contain 1.26% total dietary Lys. The ratio of TSAA to Lys was set at 0.72, and the ratio of Thr to Lys was set at 0.70 in all diets. All Exp. contained a positive control (PC) and a PC supplemented with crystalline Gly. The PC diet contained approximately 22.2% CP with no crystalline Gly or L-Lys•HCl in the diet. All diets except for the PC contained supplemental crystalline Gly to achieve 2.32% total dietary Gly + Ser, a level which previously has been shown to maximize growth performance of broilers fed low CP, AA-supplemented diets (Dean et

Table 3.1. Percentage composition of corn-soybean meal diets with incremental levels of L-Lysine•HCl in Experiment 1, as-fed basis[1]

			L-Lys•HCl (%)						
Ingredient	PC[1]	PC + Gly	0.15	0.17	0.19	0.21	0.23	0.25	0.27
Corn	53.29	53.29	57.88	58.48	59.07	59.62	60.15	60.69	61.23
Soybean meal (47.5%)	37.13	37.13	32.82	32.25	31.68	31.11	30.54	29.97	29.40
Soy oil	4.93	4.93	4.34	4.26	4.19	4.13	4.08	4.02	3.96
Monocalcium phosphate	1.52	1.52	1.55	1.55	1.56	1.56	1.57	1.57	1.57
Limestone	1.48	1.48	1.51	1.51	1.51	1.51	1.52	1.52	1.52
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin mix[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.194	0.194	0.238	0.244	0.250	0.256	0.263	0.269	0.275
L-Lys•HCl	---	---	0.150	0.170	0.190	0.210	0.230	0.250	0.270
L-Thr	0.041	0.041	0.108	0.117	0.126	0.135	0.144	0.153	0.162
Gly	---	0.265	0.428	0.450	0.472	0.494	0.515	0.537	0.559
Cornstarch	0.559	0.294	0.131	0.119	0.110	0.126	0.148	0.170	0.191
Calculated composition									
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
CP, %	22.21	22.52	21.26	21.08	20.90	20.72	20.53	20.35	20.16
Analyzed CP, %	22.17	22.39	21.31	21.36	21.57	20.97	20.86	20.46	20.74
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P, %	0.72	0.72	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Non-phytate P, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Cl, %	0.36	0.36	0.39	0.39	0.40	0.40	0.40	0.41	0.41
K, %	0.96	0.96	0.88	0.87	0.86	0.85	0.84	0.83	0.82
Lys, %	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26
Analyzed Lys, %	1.22	1.24	1.20	1.24	1.23	1.23	1.28	1.26	1.22

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Thr, %	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Gly+Ser, %	2.06	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32
Analyzed Gly+Ser, %	1.96	2.16	2.14	2.25	2.17	2.15	2.50	2.34	2.23
Total sulfur AA, %	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Val, %	1.05	1.05	0.97	0.96	0.95	0.94	0.93	0.92	0.91
Arg, %	1.49	1.49	1.36	1.34	1.32	1.30	1.29	1.27	1.25
Ile, %	0.95	0.95	0.87	0.86	0.85	0.84	0.83	0.82	0.81

[1] A basal diet was mixed to contain the minimum of all ingredients except for L-Lys•HCl, crystalline Gly, cornstarch, and additional ingredients were added as needed to each diet. The analyzed values for total Thr and total sulfur AA averaged 0.88 and 0.87 respectively. PC = positive control.

[2] Provided per kilogram of diet: copper (copper sulfate•5H_2O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate•7H_2O), 60 mg; manganese (manganese sulfate •H_2O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate•7H_2O), 44 mg; calcium (calcium carbonate), 723 mg.

[3] Contains 750,000 mg/kg of choline.

[4] Provided per kilogram of diet: vitamin A (retinyl palmitate), 2,475 µg; vitamin D_3 (cholecalciferol), 11.25 µg; vitamin E (D,L-α-tocopheryl acetate), 50 mg; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B_{12}, 0.02 mg; d-biotin, 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; d-pantothenic acid, 18.3 mg; pyridoxine (pyridoxine•HCL), 6.4 mg; riboflavin, 15 mg; thiamin (thiamin•HCL), 13.4 mg.

al., 2006). Diets were formulated to contain 3,200 kcal ME/kg, 1.0% Ca, and 0.45% non-phytate P.

The AA composition in the diets was determined after acid hydrolysis (AOAC, 2002; Method 994.12). Total sulfur AA composition in the diets was determined after performic acid oxidation followed by acid hydrolysis (AOAC, 2002; Method 994.12).

Experiment 1 was conducted to determine the effect of incremental levels of L-Lys•HCl on growth performance of broilers. Treatments contained 8 replications with 6 chickens per replicate pen. Diet 1 for Exp. 1 was the PC diet with no crystalline Gly or L-Lys•HCl. Diet 2 was the PC diet with the addition of crystalline Gly to achieve a level of 2.32% total dietary Gly + Ser. Diets 3 to 9 had L-Lys•HCl added to the diets at 0.02% increments from 0.15 to 0.27% (Table 3.1). With the increasing levels of supplemental L-Lys•HCl, the diets decreased in SBM content, which resulted in decreased CP from 22.2 to 20.2%.

Experiment 2 also was conducted to determine the effect of incremental levels of L-Lys•HCl on growth performance of broilers. Treatments contained 7 replications with 6 chickens per replicate. The treatment arrangement was identical to Exp. 1 except diets 3 to 10 had L-Lys•HCl added to the diets at 0.05% increments from 0.25 to 0.60% (Table 3.2). With the increasing levels of supplemental L-Lys•HCl, the diets decreased in SBM content, which resulted in decreased CP from 22.2 to 17.4%.

Experiment 3 was conducted to confirm the results of Exp. 1 and 2. Treatments contained 8 replications with 6 chickens per replicate. The treatment arrangement was identical to Exp. 1 and 2 except diets 3 to 5 had L-Lys•HCl added to the diets at 0.05% increments from 0.20 to 0.30% (Table 3.3). With the increasing levels of supplemental

Table 3.2. Percentage composition of corn-soybean meal diets with incremental levels of L-Lysine•HCl in Experiment 2, as-fed basis[1]

			L-Lys•HCl (%)							
Ingredient	PC[1]	PC + Gly	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60
Corn	52.97	52.97	60.47	61.88	63.30	64.72	66.13	67.54	68.97	70.26
Soybean meal (47.5%)	37.16	37.16	29.99	28.56	27.14	25.71	24.29	22.86	21.44	20.02
Soy oil	4.90	4.90	3.90	3.69	3.49	3.28	3.07	2.87	2.66	2.45
Monocalcium PO_4	1.52	1.52	1.57	1.58	1.59	1.60	1.61	1.62	1.64	1.65
Limestone	1.48	1.48	1.52	1.53	1.54	1.54	1.55	1.56	1.56	1.57
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin mix[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.194	0.194	0.269	0.284	0.300	0.315	0.330	0.345	0.360	0.376
L-Lys•HCl	---	---	0.250	0.300	0.350	0.400	0.450	0.500	0.550	0.600
L-Thr	0.041	0.041	0.153	0.176	0.198	0.221	0.243	0.266	0.288	0.311
Gly	---	0.266	0.538	0.593	0.647	0.702	0.757	0.812	0.866	0.884
Cornstarch	0.884	0.618	0.496	0.549	0.603	0.657	0.714	0.778	0.847	1.031
Calculated composition										
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
CP, %	22.19	22.50	20.39	19.97	19.55	19.12	18.71	18.27	17.85	17.38
Analyzed CP, %	21.60	22.02	19.64	19.23	19.38	18.93	18.90	18.06	17.98	18.04
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P, %	0.72	0.72	0.71	0.70	0.70	0.70	0.69	0.69	0.69	0.68
Non-phytate P, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Cl, %	0.36	0.36	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48
K, %	0.96	0.96	0.83	0.80	0.78	0.75	0.72	0.70	0.67	0.64
Lys, %	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26
Analyzed Lys, %	1.18	1.19	1.16	1.19	1.20	1.22	1.19	1.19	1.22	1.29

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Thr, %	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Gly+Ser, %	2.05	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32
Analyzed Gly+Ser, %	1.93	2.25	2.12	2.26	2.15	2.26	2.17	2.17	2.25	2.32
Total sulfur AA, %	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Val, %	1.05	1.05	0.92	0.89	0.86	0.84	0.81	0.78	0.76	0.73
Arg, %	1.49	1.49	1.27	1.22	1.18	1.13	1.09	1.05	1.00	0.96
Ile, %	0.95	0.95	0.82	0.79	0.76	0.74	0.71	0.68	0.66	0.63

[1] A basal diet was mixed to contain the minimum of all ingredients except for L-Lys•HCl, crystalline Gly, cornstarch, and additional ingredients were added as needed to each diet. The analyzed values for total Thr and total sulfur AA averaged 0.87 and 0.85 respectively. PC = positive control.

[2] Provided per kilogram of diet: copper (copper sulfate•5H_2O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate•7H_2O), 60 mg; manganese (manganese sulfate •H_2O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate•7H_2O), 44 mg; calcium (calcium carbonate), 723 mg.

[3] Contains 750,000 mg/kg of choline.

[4] Provided per kilogram of diet: vitamin A (retinyl palmitate), 2,475 µg; vitamin D_3 (cholecalciferol), 11.25 µg; vitamin E (DL-α-tocopheryl acetate), 50 mg; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B_{12}, 0.02 mg; d-biotin, .6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; d-pantothenic acid, 18.3 mg; pyridoxine (pyridoxine•HCL), 6.4 mg; riboflavin, 15 mg; thiamin (thiamin•HCL), 13.4 mg.

Table 3.3. Percentage composition of corn-soybean meal diets with incremental levels of L-Lysine•HCl in Experiment 3, as-fed basis[1]

			L-Lys•HCl (%)		
Ingredient	PC[1]	PC + Gly	0.20	0.25	0.30
Corn	52.92	52.92	59.00	60.42	61.84
Soybean meal (47.5% CP)	37.16	37.16	31.42	30.00	28.57
Soy oil	5.04	5.04	4.24	4.03	3.83
Monocalcium phosphate	1.52	1.52	1.56	1.57	1.58
Limestone	1.48	1.48	1.51	1.52	1.53
Salt	0.50	0.50	0.50	0.50	0.50
Mineral mix[2]	0.25	0.25	0.25	0.25	0.25
Choline chloride[3]	0.05	0.05	0.05	0.05	0.05
Nutra Blend vitamins[4]	0.25	0.25	0.25	0.25	0.25
DL-Met	0.195	0.195	0.254	0.269	0.285
L-Lys•HCl	---	---	0.200	0.250	0.300
L-Thr	0.041	0.041	0.131	0.153	0.176
Gly	---	0.266	0.484	0.538	0.593
Cornstarch	0.591	0.325	0.147	0.203	0.256
Calculated composition					
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200
CP, %	22.19	22.50	20.82	20.40	19.97
Analyzed CP, %	22.83	22.50	21.33	21.02	20.42
Ca, %	1.00	1.00	1.00	1.00	1.00
P, %	0.72	0.72	0.71	0.71	0.70
Non-phytate P, %	0.45	0.45	0.45	0.45	0.45
Cl, %	0.36	0.36	0.40	0.41	0.42
K, %	0.96	0.96	0.85	0.83	0.80
Lys, %	1.26	1.26	1.26	1.26	1.26
Analyzed Lys, %	1.23	1.23	1.21	1.22	1.23
Thr, %	0.88	0.88	0.88	0.88	0.88
Gly + Ser, %	2.05	2.32	2.32	2.32	2.32
Analyzed Gly+Ser, %	2.03	2.11	2.23	2.21	2.25
Total sulfur AA, %	0.91	0.91	0.91	0.91	0.91
Val, %	1.05	1.05	0.94	0.92	0.89
Arg, %	1.49	1.49	1.31	1.27	1.22
Ile, %	0.95	0.95	0.84	0.82	0.79

[1] A basal diet was mixed to contain the minimum of all ingredients except for L-Lys•HCl, crystalline Gly, cornstarch, and additional ingredients were added as needed to each diet. The analyzed values for total Thr and total sulfur AA averaged 0.89 and 0.87 respectively. PC = positive control.

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[2] Provided per kilogram of diet: copper (copper sulfate•5H_2O), 4 mg; iodine (potassium iodate), 1.0 mg; iron (ferrous sulfate•7H_2O), 60 mg; manganese (manganese sulfate•H_2O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate•7H_2O), 44 mg; calcium (calcium carbonate), 723 mg.
[3] Contains 750,000 mg/kg of choline.
[4] Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,002.78 IU; vitamin D_3 (cholecalciferol), 3003.80 IU; vitamin E (DL-α-tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B_{12}, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine•HCL), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin•HCL), 3.00 mg.

L-Lys•HCl, the diets decreased in SBM content, which resulted in decreased CP from 22.2 to 20.0%.

Experiment 4 and 5 were conducted to determine the order of limiting AA other than Met, Thr, Lys, and Gly in low CP, C-SBM diets for broiler chicks. In Exp. 4 and 5, treatments contained 7 replications with 6 chickens per replicate pen. In Exp. 4, the order of limiting AA was determined in a C-SBM diet containing 0.45% L-Lys•HCl and 17.8% CP (Table 3.4). The treatments for Exp. 4 were 1) PC with 22.2% CP and no crystalline Gly or L-Lys•HCl added; 2) PC + 0.268% crystalline Gly; 3) negative control (NC with 0.45% L-Lys•HCl); 4) NC + 0.247% Ile; 5) NC + 0.484% L-Arg•HCl; 6) NC + 0.249% Val; 7) NC + 0.247% Ile + 0.484% Arg; 8) NC + 0.247% Ile + 0.249% Val; 9) NC + 0.249% Val + 0.484% Arg; 10) NC + 0.247% Ile + 0.484% Arg + 0.249% Val. Diets 2 to 10 contained supplemental crystalline Gly to provide a total dietary Gly + Ser level of 2.32%.

It is possible that the order of limiting AA (other than Met, Thr, Lys, and Gly) changes in a diet with different levels of supplemental Lys because of the change in ratio of corn to SBM. Therefore, Exp. 5 was conducted in an identical manner to Exp. 4 except the diets with the added crystalline AA contained the same ratio of corn to SBM that is found in a diet with 0.25% L-Lys•HCl, which was achieved by dilution with cornstarch (Table 3.5). The ratio of Arg:Ile and Arg:Val changes from 1.54 and 1.35 to 1.55 to 1.38 respectively between a diet with 0.45% L-Lys•HCl to a diet with 0.25% L-Lys•HCl. Arginine relative to Ile and Val is less in a diet with 0.45% L-Lys•HCl compared with a diet with 0.25% L-Lys•HCl. The ratios of Arg:Ile at 1.55 and Arg:Val at 1.38 that are in the 0.25% L-Lys•HCl diet are the ratios that are found in the negative control diet in Exp. 5. Treatments contained 7 replications with 6 chickens per replicate. The treatments for Exp. 5 were 1) PC with 22.2% CP and no crystalline Gly or L-Lys•HCl added; 2) PC + 0.265% crystalline Gly; 3) NC; 4) NC + 0.253% Ile; 5) NC + 0.484% L-Arg•HCl; 6) NC + 0.268% Val; 7) NC + 0.253% Ile + 0.484% Arg; 8) NC + 0.253% Ile + 0.268% Val; 9) NC + 0.268% Val + 0.484% Arg; 10) NC + 0.253% Ile + 0.484% Arg + 0.268% Val. Diets 2 to 10 contained supplemental crystalline Gly to provide a total dietary Gly + Ser level of 2.32%.

For all Exp., data were analyzed by ANOVA as a completely randomized design using the GLM procedures in SAS (SAS Inst. Inc., Cary, NC). The pen of chicks was the experimental unit in all Exp. The PDIFF option of SAS was used to compare individual diets to the PC diet. For Exp. 1, 2, and 3, linear and quadratic contrasts were used.

RESULTS

In Exp. 1 (Table 3.6), broilers fed the 0.21% or 0.23% L-Lys•HCl diets had an increased ADG and GF compared with broilers fed the PC diet ($P < 0.089$). The incremental L-Lys•HCl additions resulted in no detrimental effects on growth performance of broilers. Broilers fed the PC + Gly had increased GF compared with broilers fed the PC diet ($P = 0.064$).

In Exp. 2 (Table 3.7), broilers fed the 0.30%, 0.45% and greater L-Lys•HCl levels had decreased ADG and ADFI compared with broilers fed the PC diet ($P < 0.042$). Broilers fed the 0.45% L-Lys•HCl and greater had decreased GF compared with broilers fed the PC diet ($P < 0.001$). There was a significant linear effect of L-Lys•HCl ($P < 0.001$) on ADG, ADFI, and GF in broilers fed incremental levels of L-Lys•HCl. As in Exp.

Table 3.4. Percentage composition of corn-soybean meal diets to determine the 5^{th}, 6^{th}, and 7^{th} limiting amino acids in Experiment 4, as-fed basis[1]

Ingredient	PC[1]	PC+Gly	NC	Ile	Arg	Val	Ile+Arg	Ile+Val	Arg+Val	All 3
Corn	53.25	53.13	65.17	65.17	65.17	65.17	65.17	65.17	65.17	65.17
SBM (47.5% CP)	37.14	37.15	24.37	24.37	24.37	24.37	24.37	24.37	24.37	24.37
Soy oil	5.07	5.17	3.47	3.47	3.47	3.47	3.47	3.47	3.47	3.47
Monocalcium PO_4	1.52	1.52	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62
Limestone	1.48	1.48	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Nutra Blend vitamins[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.194	0.194	0.332	0.332	0.332	0.332	0.332	0.332	0.332	0.332
L-Lys•HCl	---	---	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450
L-Thr	0.041	0.041	0.245	0.245	0.245	0.245	0.245	0.245	0.245	0.245
Gly	---	0.268	0.768	0.768	0.768	0.768	0.768	0.768	0.768	0.768
L-Ile	---	---	---	0.247	---	---	0.247	0.247	---	0.247
L-Arg•HCl	---	---	---	---	0.484	---	0.484	---	0.484	0.484
L-Val	---	---	---	---	---	0.249	---	0.249	0.249	0.249
Cornstarch	0.268	---	0.984	0.737	0.500	0.735	0.253	0.488	0.251	0.004
Calculated composition										
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
CP, %	22.20	22.20	17.79	17.96	18.60	17.98	18.76	18.14	18.78	18.95
Analyzed CP, %	21.95	22.63	18.71	18.91	19.35	18.72	19.32	19.31	19.56	19.89
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P, %	0.72	0.72	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Non-phytate P, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Cl, %	0.36	0.36	0.45	0.45	0.53	0.45	0.53	0.45	0.53	0.53

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K, %	0.96	0.96	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Lys, %	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26
Analyzed Lys, %	1.20	1.25	1.28	1.27	1.26	1.20	1.26	1.25	1.23	1.26
Thr, %	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Gly+Ser, %	2.06	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32
Analyzed Gly+Ser, %	1.93	2.26	2.22	2.24	2.21	2.14	2.23	2.16	2.14	2.21
Total sulfur AA, %	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Val, %	1.05	1.05	0.81	0.81	0.81	1.05	0.81	1.05	1.05	1.05
Analyzed Val, %	0.99	1.02	0.79	0.77	0.77	1.00	0.75	1.03	1.00	1.02
Arg, %	1.49	1.49	1.09	1.09	1.49	1.09	1.49	1.09	1.49	1.49
Analyzed Arg, %	1.45	1.51	1.12	1.07	1.48	1.09	1.49	1.14	1.43	1.47
Ile, %	0.95	0.95	0.71	0.95	0.71	0.71	0.95	0.95	0.71	0.95
Analyzed Ile, %	0.91	0.94	0.71	0.95	0.69	0.69	0.92	0.94	0.73	0.94
Trp, %	0.27	0.27	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phe + Tyr, %	1.90	1.90	1.44	1.44	1.44	1.44	1.44	1.44	1.44	1.44
His, %	0.60	0.60	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Leu, %	1.89	1.89	1.54	1.54	1.54	1.54	1.54	1.54	1.54	1.54
Thr:Lys	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Gly+Ser:Lys	1.63	1.84	1.84	1.84	1.84	1.84	1.84	1.84	1.84	1.84
TSAA:Lys	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Val:Lys	0.83	0.83	0.64	0.64	0.64	0.83	0.64	0.83	0.83	0.83
Arg:Lys	1.18	1.18	0.86	0.86	1.18	0.86	1.18	0.86	1.18	1.18
Ile:Lys	0.75	0.75	0.56	0.75	0.56	0.56	0.75	0.75	0.56	0.75
Trp:Lys	0.22	0.22	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Phe+Tyr:Lys	1.51	1.51	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15
Ile:Arg	0.64	0.64	0.65	0.87	0.48	0.65	0.64	0.87	0.48	0.64
Val:Arg	0.71	0.71	0.74	0.74	0.54	0.96	0.54	0.96	0.70	0.70
Arg:Ile	1.57	1.57	1.54	1.15	2.10	1.54	1.57	1.15	2.10	1.57
Arg:Val	1.42	1.42	1.35	1.35	1.84	1.04	1.84	1.04	1.42	1.42

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[1] A basal diet was mixed to contain the minimum of all ingredients except for L-Lys•HCl, crystalline Gly, L-Ile, L-Val, L-Arg•HCl, cornstarch, and additional ingredients were added as needed to each diet. The analyzed values for total Thr and total sulfur AA averaged 0.88 and 0.84 respectively. PC = positive control.
[2] Provided per kilogram of diet: copper (copper sulfate•5H_2O), 4 mg; iodine (potassium iodate), 1.0 mg; Iron (ferrous sulfate•7H_2O), 60 mg; manganese (manganese sulfate •H_2O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate•7H_2O), 44 mg; calcium (calcium carbonate), 723 mg.
[3] Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,002.78 IU; vitamin D_3 (cholecalciferol), 3003.80 IU; vitamin E (DL-α-tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B_{12}, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine•HCL), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin•HCL), 3.00 mg.
[4] Contains 750,000 mg/kg of choline.

Table 3.5. Percentage composition of corn-soybean meal diets to determine the 5th, 6th, and 7th limiting amino acids in Experiment 5, as-fed basis[1]

Ingredient	PC[1]	PC+Gly	NC	Ile	Arg	Val	Ile+Arg	Ile+Val	Arg+Val	All 3
Corn	53.51	53.13	52.00	52.00	52.00	52.00	52.00	52.00	52.00	52.00
SBM (47.5% CP)	37.11	37.15	25.79	25.79	25.79	25.79	25.79	25.79	25.79	25.79
Soy oil	5.09	5.17	2.40	2.40	2.40	2.40	2.40	2.40	2.40	2.40
Monocalcium PO_4	1.52	1.52	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Limestone	1.48	1.48	1.53	1.53	1.53	1.53	1.53	1.53	1.53	1.53
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Nutra Blend vitamins[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.193	0.194	0.360	0.360	0.360	0.360	0.360	0.360	0.360	0.360
L-Lys•HCl	---	---	0.439	0.439	0.439	0.439	0.439	0.439	0.439	0.439
L-Thr	0.041	0.041	0.257	0.257	0.257	0.257	0.257	0.257	0.257	0.257
Gly	---	0.265	0.788	0.788	0.788	0.788	0.788	0.788	0.788	0.788
L-Ile	---	---	---	0.253	---	---	0.253	0.253	---	0.253
L-Arg•HCl	---	---	---	---	0.484	---	0.484	---	0.484	0.484
L-Val	---	---	---	---	---	0.268	---	0.268	0.268	0.268
Cornstarch	---	---	13.726	13.473	13.242	13.458	12.989	13.205	12.974	12.721
Calculated composition										
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
CP, %	22.21	22.51	18.38	18.55	19.18	18.57	19.35	18.74	19.38	19.47
Analyzed CP, %	22.32	23.14	18.54	18.91	19.28	19.06	19.87	18.13	19.30	19.87
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P, %	0.72	0.72	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Non-phytate P, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Cl, %	0.36	0.36	0.45	0.45	0.53	0.45	0.53	0.45	0.53	0.53

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K, %	0.96	0.96	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Lys, %	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26
Analyzed Lys, %	1.20	1.28	1.22	1.26	1.24	1.29	1.27	1.24	1.26	1.27
Thr, %	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Gly + Ser, %	2.06	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32	2.32
Analyzed Gly+Ser, %	1.98	2.27	2.25	2.27	2.23	2.30	2.24	2.26	2.22	2.25
Total sulfur AA, %	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Val, %	1.05	1.05	0.79	0.79	0.79	1.05	0.79	1.05	1.05	1.05
Analyzed Val, %	0.99	1.05	0.80	0.79	0.73	1.08	0.81	1.03	1.03	1.06
Arg, %	1.49	1.49	1.09	1.09	1.49	1.09	1.49	1.09	1.49	1.49
Analyzed Arg, %	1.51	1.53	1.12	1.13	1.49	1.14	1.51	1.03	1.49	1.42
Ile, %	0.95	0.95	0.70	0.95	0.70	0.70	0.95	0.95	0.70	0.95
Analyzed Ile, %	0.92	0.96	0.73	0.95	0.66	0.73	0.96	0.94	0.71	0.97
Trp, %	0.27	0.27	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phe + Tyr, %	1.91	1.90	1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42
His, %	0.60	0.60	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Leu, %	1.89	1.89	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46
Thr:Lys	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Gly+Ser:Lys	1.63	1.84	1.84	1.84	1.84	1.84	1.84	1.84	1.84	1.84
TSAA:Lys	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Val:Lys	0.83	0.83	0.63	0.63	0.63	0.83	0.63	0.83	0.83	0.83
Arg:Lys	1.18	1.18	0.86	0.86	1.18	0.86	1.18	0.86	1.18	1.18
Ile:Lys	0.76	0.75	0.56	0.75	0.56	0.56	0.75	0.75	0.56	0.75
Trp:Lys	0.22	0.22	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Phe+Tyr:Lys	1.52	1.51	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13
Ile:Arg	0.64	0.64	0.64	0.87	0.47	0.64	0.64	0.87	0.47	0.64
Val:Arg	0.70	0.70	0.72	0.72	0.53	0.96	0.53	0.96	0.70	0.70
Arg:Ile	1.57	1.57	1.56	1.15	2.13	1.56	1.57	1.15	2.13	1.57
Arg:Val	1.42	1.42	1.38	1.38	1.89	1.04	1.89	1.04	1.42	1.42

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[1] A basal diet was mixed to contain the minimum of all ingredients except for L-Lys•HCl, crystalline Gly, L-Ile, L-Val, L-Arg•HCl, cornstarch, and additional ingredients were added as needed to each diet. The analyzed values for total Thr and total sulfur AA averaged 0.87 and 0.83 respectively. PC = positive control.
[2] Provided per kilogram of diet: copper (copper sulfate•$5H_2O$), 4 mg; iodine (potassium iodate), 1.0 mg; Iron (ferrous sulfate•$7H_2O$), 60 mg; manganese (manganese sulfate •H_2O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate•$7H_2O$), 44 mg; calcium (calcium carbonate), 723 mg.
[3] Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,002.78 IU; vitamin D_3 (cholecalciferol), 3003.80 IU; vitamin E (DL-α-tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B_{12}, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine•HCL), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin•HCL), 3.00 mg.
[4] Contains 750,000 mg/kg of choline.

1, broilers fed the PC + Gly had increased GF compared with broilers fed the PC diet (*P* < 0.010).

In Exp. 3 (Table 3.8), broilers fed the 0.20 and 0.25% L-Lys•HCl diets had increased ADG compared with broilers fed the PC diet (*P* = 0.067). Broilers fed 0.30% L-Lys•HCl diet had increased ADFI compared with broilers fed the PC diet (*P* < 0.093). There was a significant decreasing linear effect of L-Lys•HCl (*P* < 0.065) on GF in broilers fed incremental levels of L-Lys•HCl. Also, broilers fed the PC + Gly had increased ADG and ADFI compared with broilers fed the PC diet (*P* < 0.007).

In Exp. 4 (Table 3.9), broilers fed the NC with the addition of Arg and Val or the NC with the addition of all 3 crystalline AA had increased ADG, ADFI, and GF (*P* < 0.080) compared with broilers fed the NC. Broilers fed the PC or the PC + Gly had increased ADG, ADFI, and GF (*P* < 0.011) compared with broilers fed the NC diet. Broilers fed the NC, NC + Ile, NC + Arg, NC + Val, NC + Ile + Arg, and NC + Ile + Val had significantly lower ADG and GF (*P* < 0.004) compared with broilers fed the PC, but only broilers fed the NC + Ile or the NC + Val had decreased ADFI compared with broilers fed the PC.

In Exp. 5 (Table 3.10), broilers fed the NC + Val +Arg had increased ADG (*P* < 0.035) compared with broilers fed the NC. Broilers fed the NC + all 3 crystalline AA had increased ADG and GF (*P* < 0.022) compared with broilers fed the NC. Broilers fed the PC or PC + Gly had increased ADG, ADFI, and GF (*P* < 0.059) compared with broilers fed the NC. Broilers fed the NC or NC + individual or any combination of the 3 crystalline AA had decreased ADG and GF (*P* < 0.034) compared with broilers fed the PC, except the GF for broilers fed the NC + all 3 crystalline AA had the same GF as

Table 3.6. Growth performance of broiler chicks fed incremental levels of L-Lysine•HCl in Experiment 1[1]

Treatment	CP, %	ADG, g	ADFI, g	GF, g/g
1) Positive control (PC)	22.21	30.51	39.19	0.778
2) PC + Gly[2]	22.52	31.30	39.23	0.798[a]
3) 0.15% L-Lys•HCl	21.26	31.73	40.23	0.789
4) 0.17% L-Lys•HCl	21.08	30.89	39.44	0.783
5) 0.19% L-Lys•HCl	20.90	31.82	40.28	0.790
6) 0.21% L-Lys•HCl	20.72	32.39[a]	40.67	0.797[a]
7) 0.23% L-Lys•HCl	20.53	32.05[a]	39.99	0.802[a]
8) 0.25% L-Lys•HCl	20.35	31.93	40.34	0.791
9) 0.27% L-Lys•HCl	20.16	31.33	39.74	0.789
	SEM	0.628	0.737	0.007
	P-value	0.525	0.839	0.416
	Linear	0.738	0.988	0.474
	Quadratic	0.315	0.592	0.263

[1] Data are means of 8 replicates of 6 broilers per replicate.
[2] Crystalline Gly was added to each diet to achieve 2.32% Gly + Ser (Dean et al., 2006) except for treatment 1 (positive control).
[a] Significantly different (*P* < 0.10) from the positive control.

Table 3.7. Growth performance of broiler chicks fed incremental levels of L-Lysine•HCl in Experiment 2[1]

Treatment	CP, %	ADG, g	ADFI, g	GF, g/g
1) Positive control (PC)	22.19	27.67	37.08	0.745
2) PC + Gly[2]	22.50	28.87	37.38	0.772[a]
3) 0.25% L-Lys•HCl	20.39	28.04	37.18	0.754
4) 0.30% L-Lys•HCl	19.97	25.81[a]	34.55[a]	0.747
5) 0.35% L-Lys•HCl	19.55	26.87	36.35	0.740
6) 0.40% L-Lys•HCl	19.12	26.45	36.06	0.734
7) 0.45% L-Lys•HCl	18.71	24.12[a]	34.08[a]	0.707[a]
8) 0.50% L-Lys•HCl	18.27	23.64[a]	33.92[a]	0.697[a]
9) 0.55% L-Lys•HCl	17.85	24.02[a]	34.34[a]	0.699[a]
10) 0.60% L-Lys•HCl	17.38	22.85[a]	33.45[a]	0.683[a]
	SEM	0.633	0.796	0.007
	P-value	0.001	0.001	0.001
	Linear	0.001	0.001	0.001
	Quadratic	0.838	0.862	0.903

[1] Data are means of 7 replicates of 6 broilers per replicate.
[2] Crystalline Gly was added to each diet to achieve 2.32% Gly + Ser (Dean et al., 2006) except for treatment 1 (positive control).
[a] Significantly different (*P* < 0.10) from the positive control.

Table 3.8. Growth performance of broiler chicks fed incremental levels of L-Lysine•HCl in Experiment 3[1]

Treatment	CP, %	ADG, g	ADFI, g	GF, g/g
1) Positive control (PC)	22.19	30.57	38.18	0.803
2) PC + Gly[2]	22.50	33.55[a]	41.64[a]	0.805
3) 0.20% L-Lys•HCl	20.82	32.41[a]	39.85	0.814
4) 0.25% L-Lys•HCl	20.40	32.27[a]	39.90	0.810
5) 0.30% L-Lys•HCl	19.97	31.41	40.26[a]	0.781
	SEM	0.637	0.851	0.012
	P-value	0.028	0.100	0.372
	Linear	0.276	0.735	0.065
	Quadratic	0.641	0.881	0.415

[1] Data are means of 8 replicates of 6 broilers per replicate.
[2] Crystalline Gly was added to each diet to achieve 2.32% Gly + Ser (Dean et al., 2006) except for treatment 1 (positive control).
[a] Significantly different (*P* < 0.10) from positive control.

Table 3.9. Determination of the 5th, 6th, and 7th limiting amino acids in a low crude protein, corn-soybean meal diet in Experiment 4[1]

Treatment	ADG, g	ADFI, g	GF, g/g
1) Positive control (PC)	29.52[b]	37.04[b]	0.797[b]
2) PC+ Gly[2]	29.96[b]	38.04[b]	0.788[b]
3) Negative control (NC) + Gly	24.67[a]	33.46[a]	0.737[a]
4) NC + Gly + Ile	24.36[a]	32.76[a]	0.743[a]
5) NC + Gly + Arg	26.31[a]	35.35	0.744[a]
6) NC + Gly + Val	24.63[a]	34.15[a]	0.723[a]
7) NC + Gly + Ile + Arg	26.30[a]	35.22	0.747[a]
8) NC + Gly + Ile + Val	25.63[a]	34.79	0.736[a]
9) NC + Gly + Val + Arg	27.84[b]	35.89[b]	0.776[a,b]
10) NC + Gly + Ile + Val + Arg	28.72[b]	36.35[b]	0.790[b]
SEM	0.762	0.964	0.008
P-value	0.001	0.008	0.001

[1] Data are means of 7 replicates of 6 broilers per replicate.
[2] Crystalline Gly was added to each diet to achieve 2.32% Gly + Ser (Dean et al., 2006) except for treatment 1 (positive control).
[a] Significantly different (*P* < 0.10) from positive control.
[b] Significantly different (*P* < 0.10) from the negative control.

Table 3.10. Determination of the 5th, 6th, and 7th limiting amino acids in a low crude protein, corn-soybean meal diet in Experiment 5[1]

Treatment	ADG, g	ADFI, g	GF, g/g
1) Positive control (PC)	28.88[b]	37.36[b]	0.775[b]
2) PC + Gly[2]	28.75[b]	37.35[b]	0.770[b]
3) Negative control (NC) + Gly	24.74[a]	35.06[a]	0.706[a]
4) NC + Gly + Ile	24.90[a]	34.65[a]	0.718[a]
5) NC + Gly + Arg	25.20[a]	36.13	0.698[a]
6) NC + Gly + Val	25.06[a]	35.87	0.700[a]
7) NC + Gly + Ile + Arg	24.58[a]	34.52[a]	0.712[a]
8) NC + Gly + Ile + Val	25.14[a]	35.19[a]	0.715[a]
9) NC + Gly + Val + Arg	26.71[a,b]	36.79	0.726[a]
10) NC + Gly + Ile + Val + Arg	26.89[a,b]	34.71[a]	0.775[b]
SEM	0.648	0.840	0.013
P-value	0.001	0.103	0.001

[1] Data are means of 7 replicates of 6 broilers per replicate.
[2] Crystalline Gly was added to each diet to achieve 2.32% Gly + Ser (Dean et al., 2006) except for treatment 1 (positive control).
[a] Significantly different ($P < 0.10$) from positive control.
[b] Significantly different ($P < 0.10$) from the negative control.

broilers fed the PC. Broilers fed the NC, NC + Ile, NC + Ile + Arg, NC + Ile + Val, or the NC + all 3 crystalline AA had decreased ADFI ($P < 0.072$) compared with broilers fed the PC.

DISCUSSION

The objective of Exp. 1 was to determine the highest level of L-Lys•HCl that could be included in C-SBM diets for broilers that does not reduce growth performance when crystalline DL-Met, L-Thr, Lys, and Gly were supplemented to the diet. Average daily gain and GF for broilers fed the 0.21% and 0.23% L-Lys•HCl diets was greater than the ADG and GF of the broilers fed the positive control; however, there were no significant negative effects on ADG, ADFI, or GF with the incremental levels of L-Lys•HCl.

Because there were no negative effects of L-Lys•HCl supplementation on growth performance in Exp. 1, Exp. 2 was conducted with greater incremental levels of L-

Lys•HCl. The incremental levels of L-Lys•HCl had a significant linear effect on ADG, ADFI, and GF. Broilers fed the 0.30% L-Lys•HCl diet had decreased ADG and ADFI; therefore, at this level of L-Lys•HCl inclusion, we believe that there are AA that are becoming limiting in this diet. Based on the ADG, ADFI, and GF, broilers fed the 0.25% L-Lys•HCl diet had optimal growth performance. Although diets containing 0.25, 0.30, 0.35, or 0.40% L-Lys•HCl were calculated to be deficient in Arg, Val, and Ile, these three crystalline AA were not supplemented in the diet. Diets containing 0.45 or 0.50% L-Lys•HCl were calculated to be deficient in Val, Arg, Ile, and Trp. The 0.55% L-Lys•HCl diet was calculated to be deficient in Val, Arg, Ile, Trp, and His. The 0.60% L-Lys•HCl diet was calculated to be deficient in Val, Arg, Ile, Trp, His, and Phe. Although these diets were calculated to be deficient in these AA, cornstarch was added to the diet in place of them. The decrease in growth performance of broilers fed the greater than 0.45% L-Lys•HCl diets could be due to the multiple AA that are considered to be limiting in these diets. Although the 0.25% L-Lys•HCl diet was calculated to be deficient in Arg, Val, and Ile, the results of Exp. 2 indicate that 0.25% L-Lys•HCl can be added to C-SBM diets supplemented with DL-Met, L-Thr, and Gly with no negative effects on growth performance, and that at 0.30% added L-Lys•HCl an AA other than these four AA becomes limiting.

The third Exp. was conducted to confirm that 0.25% L-Lys•HCl could be added to broiler diets with no detrimental effects on growth performance. Our results suggest that supplementation of 0.30% L-Lys•HCl or greater in a C-SBM diet (also supplemented with DL-Met, L-Thr, and Gly) will decrease growth performance of broilers. Experiment 3 confirmed our results of Exp. 2 that a C-SBM with addition of 0.25% L-Lys•HCl could be fed to broilers without negative effects on growth performance as long as DL-Met, L-Thr, and Gly are supplemented in the diet.

Experiments 4 and 5 were conducted to determine the order of fifth, sixth, and seventh limiting AA in low CP, C-SBM diets. When Val, Arg, and Ile were all added back to the NC, ADG and GF were similar to the level of the broilers fed the PC. The increased growth response of broilers fed the NC + Arg + Val in Exp. 4 indicate that the order of AA limitation in a C-SBM diet containing 0.45% L-Lys•HCl (and DL-Met, L-Thr, and Gly) was Arg and Val equally limiting and Ile as the next limiting AA.

Based on Exp. 1, 2, and 3, a diet supplemented with 0.25% L-Lys•HCl and with DL-Met, Thr, and Gly resulted in maximum growth performance relative to a conventional diet. Because the corn to SBM ratio will be different in a diet containing 0.25% L-Lys•HCl relative to a diet containing 0.45% L-Lys•HCl, the order of limiting AA could change. The ratio of Arg to Ile and Arg to Val changes in a diet with 0.25% L-Lys•HCl compared with a diet containing 0.45% L-Lys•HCl. There is less Arg relative to Ile and Val in a diet supplemented with 0.45% L-Lys•HCl than in a diet with 0.25% L-Lys•HCl. Therefore, Exp. 5 was conducted in a similar manner to Exp. 4 except the diets with added AA contained the same ratio of C-SBM that is found in a diet with 0.25% L-Lys•HCl, which was achieved by dilution with cornstarch. When Val, Arg, and Ile were all added back to the NC, ADG and GF was similar to the level of the broilers fed the PC. Based on the increased ADG of broilers fed the NC + Val + Arg, the results of Exp. 5 indicate that Arg and Val may be equally limiting (after Met, Thr, Lys, and Gly) in a diet containing 0.25% L-Lys•HCl.

Corzo et al. (2005) reported that broilers fed a corn and SBM diet with 0.61% L-Lys•HCl (approximately 18.0% CP) and supplemental Gly or L-Leu had FCR equal to broilers fed the control diet (approximately 22% CP). However in our three L-Lys•HCl titration Exp., broilers fed a C-SBM diet with 0.45% L-Lys•HCl (approximately 18.7% CP) had decreased GF compared with broilers fed the control diet (approximately 22.2% CP).

Dean et al. (2006) reported that broilers fed low CP, AA-supplemented diets had growth performance equal to broilers fed conventional C-SBM diets as long as crystalline Gly was supplemented. However, the 16% CP diet was not considered practical because it contained additional crystalline AA other than the commercially available AA. Our research indicates that 0.25% L-Lys•HCl can be supplemented in a C-SBM diet for broilers without negatively affecting growth performance as long as supplemental DL-Met, L-Thr, and Gly are added to the diet.

Bregendahl et al. (2002) reported that low CP, C-SBM diets with 20.27% CP and 0.43% L-Lys•HCl to as low as 18.3% CP and 0.30% L-Lys•HCl did not improve broiler growth performance to level of the control diet; however, our research suggests that a level of 0.25% L-Lys•HCl can be included in the diet to decrease the SBM content and CP level, but broilers can still achieve growth performance equivalent to the control diet with the supplementation of crystalline Gly in this diet.

The level of total Lys in the diets used in this study was 1.26%, which may be at or below the requirement for Ross × Ross 708 broilers. If the 1.26% Lys is below the requirement, then the actual level of L-Lys•HCl that can be added may change. Experiments 4 and 5 suggest that Val and Arg are equally limiting growth performance of broilers fed diets containing greater than 0.25% L-Lys•HCl. The concentrations of these AA in the 0.25% L-Lys•HCl diet were 0.97 and 1.32, respectively. The ratio of Arg:Lys and Val:Lys in the 0.25% L-Lys•HCl diet were 1.05 and 0.77, respectively. Thus, if one sets these minimums in diet formulation, growth performance should not be affected.

Our results in Exp. 4 and 5 are similar to Han et al. (1992) who reported that the limiting AA in a 19% CP (21% total CP) diet fed to 1 to 3 wk old broilers were Met, Lys, Arg, Val, Thr, and a nitrogen source such as Glu. They reported that Met was the first limiting AA in the 19% CP diet and Lys was the second, but beyond those two, the order of limitation was not suggested. However, Han et al. (1992) did not agree necessarily with our suggestion that Gly is the fourth limiting AA in a low CP diet fed to broiler chicks, but their results suggest that a NEAA nitrogen source is required in a low CP diet.

In conclusion, supplementation of 0.25% L-Lys•HCl to a C-SBM will support broiler growth performance equivalent to broilers fed a control or commercial C-SBM diet with 22% CP as long as crystalline DL-Met, Thr, and Gly are supplemented. Arginine and Val are equal limiting after Met, Lys, Thr, and Gly in a diet with 0.25% L-Lys•HCl, in which the absence of these equally limiting AA results in a decrease in growth performance of broilers fed above the 0.25% L-Lys•HCl.

CHAPTER 4

THE GLYCINE REQUIREMENT OF BROILER CHICKS FED A LOW CRUDE PROTEIN, CORN-SOYBEAN MEAL DIET

INTRODUCTION

Researchers have shown that feeding low CP diets to broilers will decrease nitrogen excretion; therefore, reducing nitrogen loss to the environment (Aletor et al. 2000). Researchers also have shown decreased growth performance of broilers fed low CP, AA-supplemented diets (Fancher and Jensen, 1989a,b and Pinchasov et al., 1990).

Attempts to improve broiler growth performance when feeding low CP diets include modification in DEB or more specifically addition of potassium carbonate to the diet (Fancher and Jensen, 1989a,b; Han et al., 1992; Si et al., 2004), addition of NSN as triammonium citrate or L-Glu (Fancher and Jensen, 1989a,b; Pinchasov et al., 1990; Bregendahl et al., 2002), addition of EAA to meet or exceed the NRC requirements (Fancher and Jensen, 1989a,b; Bregendahl et al., 2002; Pinchasov et al., 1990), or addition of crystalline NEAA (Bregendahl et al., 2002). Neither the DEB nor the NSN modifications have been successful in resolving the negative growth performance effects of low CP, AA-supplemented diets fed to broilers (Aftab et al., 2006). However, EAA and NEAA supplementation in low CP diets have been shown to be relatively successful. Supplementation of most NEAA to low CP diets have been mainly added to make low CP diets isonitrogenous to the control diets to evaluate whether the response in growth performance was due to NSN or to a specific NEAA. However, Corzo et al. (2005) reported that broilers fed an 18% CP diet with supplemental Gly or L-Leu had FCR equal to that of broilers fed the control diet. Corzo et al. (2004) also suggested that the Gly + Ser requirement in a low CP diet should be 1.80%, which is higher than the NRC suggested Gly + Ser requirement for a 0 to 3 wk old chick. Dean et al. (2006) reported that no less than 2.32% Gly + Ser should be in a low CP diet fed to broiler chicks. Dean et al. (2006) also reported that broilers fed low CP, AA-supplemented diets had growth performance equal to broilers fed conventional C-SBM diets as long as crystalline Gly was supplemented. However, this 16% CP diet was not considered practical because it contained additional crystalline AA other than the commercially available AA. Previously (see chapter 3), a low CP, C-SBM diet supplemented with 0.25% L-Lys•HCl, crystalline DL-Met, and L-Thr along with crystalline Gly could be fed to broilers with no detrimental effects on growth performance. Therefore, the objective of this research was to determine the Gly + Ser requirement in this 0.25% L-Lys•HCl diet that achieved optimal growth performance of broilers.

MATERIALS AND METHODS

Three Exp. were conducted with male Ross x Ross broilers. On d 0 post-hatching, broilers were weighed, wing banded, and randomly allotted to treatments. Broilers were housed in temperature-controlled Petersime starter batteries with continuous fluorescent lighting. Each Exp. was conducted for 18 d. Feed in mash form and water were available on an ad libitum basis. Chicks and feeders were weighed on d 0 and 18 for the determination of growth performance. All experimental animal use was in compliance with the LSU Agricultural Center Animal Care and Use Committee.

Diets were C-SBM based and formulated to contain 1.26% total dietary Lys. The ratio of TSAA to Lys was set at 0.72, and the ratio of Thr to Lys was set at 0.70 in all diets. All Exp. contained a PC that contained approximately 22.2% CP with no crystalline Gly or L-Lys•HCl in the diet. All diets were formulated to contain 3,200 kcal ME/kg, 1.0% Ca, and 0.45%non-phytate P.

The AA composition in the diets was determined after acid hydrolysis (AOAC, 2000; Method 994.12). Total sulfur AA composition in the diets was determined after performic acid oxidation followed by acid hydrolysis (AOAC, 2000; Method 994.12).

Experiment 1 was conducted to determine the Gly requirement in a low CP, C-SBM diet with 0.25% L-Lys•HCl. Ross x Ross 708 broilers were used. They are a slow growing, high breast yield broiler mainly used for deboning and cuts-ups (Aviagen, 2007). Experiment 2 was conducted in an identical manner to Exp.1 except male Ross x Ross 308 broilers were used. The Ross x Ross 308 broiler is a fast-growing, multi-purpose broiler (Aviagen, 2007). Experiment 3 was conducted in an identical manner to Exp. 1. For all Exp., treatments contained 7 replications with 6 chickens per replicate pen. Diet 1 was the PC diet with no crystalline Gly or L-Lys•HCl. Diets 2 to 8 contained 0.25% L-Lys•HCl and added crystalline Gly in 0.125% increments from 0 to 0.75%(Table 4.1). Diet 9 contained no crystalline Gly and formulated to be isonitrogenous to diet 8 by containing 1.47% Glu.

For all Exp., data were analyzed by ANOVA as a completely randomized design using the GLM procedures in SAS (SAS Inst. Inc., Cary, NC). The pen of chicks was the experimental unit in all Exp. Using the PDIFF option of SAS, five contrasts were used to analyze diet comparisons of interest: 1.47% Glu diet vs. PC diet, 0% Gly diet, and 0.75% Gly diet and the PC diet vs. 0% or 0.75% Gly diet. For all Exp., linear and quadratic contrasts were used, and they also were analyzed by broken-line analysis

Table 4.1. Percentage composition of broiler diets with incremental levels of crystalline glycine in Experiments 1, 2, and 3, as-fed basis[1]

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Corn	52.04	58.61	58.61	58.61	58.61	58.61	58.61	58.61	58.61
Soybean meal(47.5% CP)	37.24	30.15	30.15	30.15	30.15	30.15	30.15	30.15	30.15
Soy oil	4.96	4.20	4.20	4.20	4.20	4.20	4.20	4.20	4.20
Monocalcium phosphate	1.52	1.58	1.58	1.58	1.58	1.58	1.58	1.58	1.58
Limestone	1.48	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Nutra-Blend vitamins[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met	0.197	0.274	0.274	0.274	0.274	0.274	0.274	0.274	0.274
L-Lys•HCl	---	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
L-Thr	0.043	0.156	0.156	0.156	0.156	0.156	0.156	0.156	0.156
Gly	---	---	0.125	0.250	0.375	0.500	0.625	0.750	---
Glu	---	---	---	---	---	---	---	---	1.470
Cornstarch	1.470	2.220	2.095	1.970	1.845	1.720	1.595	1.470	---
<u>Calculated nutrient composition</u>									
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
CP, %	22.16	19.70	19.85	19.99	20.14	20.28	20.43	20.58	20.58
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P, %	0.72	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Non-phytate P, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Cl, %	0.36	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
K, %	0.96	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Lys, %	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26	1.26
Thr, %	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88

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Gly+Ser, %	2.05	1.78	1.90	2.03	2.15	2.28	2.40	2.53	1.78
Analyzed Gly+Ser, %[5]	2.03	1.77	1.85	1.99	2.15	2.18	2.25	2.50	1.79
Analyzed Gly+Ser, %[6]	2.03	1.71	1.79	2.01	2.08	2.17	2.29	2.42	1.82
Analyzed Gly+Ser, %[7]	1.99	1.75	1.84	1.98	2.11	2.23	2.32	2.41	1.79
Total sulfur AA, %	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Val, %	1.05	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Arg, %	1.49	1.27	1.27	1.27	1.27	1.27	1.27	1.27	1.27
Ile, %	0.95	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Trp, %	0.27	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Phe+Tyro, %	1.90	1.65	1.65	1.65	1.65	1.65	1.65	1.65	1.65
His, %	0.60	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
Leu, %	1.88	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68

[1] A basal diet was mixed for diets 2 through 9 for experiment (Exp.) 2 and 3 to contain the minimum of all ingredients except for crystalline Gly, L-Glu, and cornstarch were added as needed to each diet. The analyzed values for total Lys, total Thr, and total sulfur amino acids (AA) averaged 1.23, 0.88, and 0.85, respectively.

[2] Provided per kilogram of diet: copper (copper sulfate•5H_2O), 4 mg; iodine (potassium iodate), 1.0 mg; Iron (ferrous sulfate•7H_2O), 60 mg; manganese (manganese sulfate •H_2O), 60 mg; selenium (sodium selenite), .1 mg; zinc (zinc sulfate•7H_2O), 44 mg; calcium (calcium carbonate), 723 mg.

[3] Contains 750,000 mg/kg of choline.

[4] Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,002.78 IU; vitamin D_3 (cholecalciferol), 3003.80 IU; vitamin E (DL-α-tocopheryl acetate), 25.00 IU; menadione (menadione sodium bisulfite), 1.50 mg; vitamin B_{12}, 0.02 mg; d-biotin, 0.10 mg; folacin (folic acid), 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine (pyridoxine•HCL), 4.00 mg; riboflavin, 10.00 mg; thiamin (thiamin•HCL), 3.00 mg.

[5] Analyzed Gly + Ser values for Exp. 1.

[6] Analyzed Gly + Ser values for Exp. 2.

[7] Analyzed Gly + Ser values for Exp. 3.

using the NLIN procedure of SAS to estimate the Gly requirement (Robbins et al., 2006).

RESULTS

Experiment 1 was conducted to determine the Gly requirement in a low CP, C-SBM diet with 0.25% L-Lys•HCl, a level which previously has been shown to maximize growth performance of male Ross x Ross 708 broilers. The incremental crystalline Gly additions had no significant linear or quadratic effect on ADG or ADFI; however, GF increased linearly ($P < 0.10$) with crystalline Gly additions (Table 4.2 and Figure 4.1). The addition of Glu did not affect (P > 0.10) ADG, ADFI, or GF compared with broilers fed the 0% Gly diet. Broilers fed the 1.47% Glu diet tended to have a higher ADFI ($P = 0.12$) and decreased GF ($P < 0.01$) compared with broilers fed the 0.750% Gly diet. Broilers fed the 1.47% Glu diet had a significantly higher ADFI ($P < 0.06$) and decreased GF ($P < 0.08$) compared with broilers fed the PC. Broilers fed the 0% Gly diet had decreased GF ($P < 0.08$) compared with broilers fed the PC. Broilers fed the 0.750% Gly diet had increased GF ($P < 0.02$) compared with broilers fed the PC. Broken-line analysis was unable to estimate a Gly requirement

Experiment 2 was conducted in an identical manner to Exp.1 except Ross x Ross 308 broilers were used. The incremental crystalline Gly additions had no significant linear or quadratic effect on ADG; however, ADFI decreased linearly ($P < 0.05$) and GF increased linearly and quadratically (P < 0.08) as Gly increased (Table 4.3). The addition of Glu did not affect (P > 0.10) ADG or ADFI compared with broilers fed the 0% Gly added diets except for GF, which was increased compared with broilers fed the 0% Gly diet (P < 0.01). Broilers fed the 1.47% Glu diet had a significantly higher ADFI ($P <$

0.10) and decreased GF (*P* < 0.01) compared with broilers fed the 0.750% Gly diet. Broilers fed the 1.47% Glu diet had ADG and ADFI that was not different compared with broilers fed the PC; however, GF decreased (*P* < 0.01) compared with broilers fed the PC. Broilers fed the 0% Gly diet had increased ADFI (*P* < 0.03) and decreased GF (*P* < 0.01) compared with broilers fed the PC. Broilers fed the 0.750% Gly diet had similar ADG, ADFI, and GF compared with broilers fed the PC. A single slope, one breakpoint analysis of GF estimated the requirement to be 2.085% total Gly + Ser or 0.309% crystalline Gly.

Experiment 3 was conducted in an identical manner to Exp.1. The incremental crystalline Gly additions had no significant linear or quadratic effect on ADG or ADFI; however, GF tended to linearly increase (*P* = 0.11) with Gly additions (Table 4.5). The addition of Glu did not affect (P > 0.10) ADG, ADFI, or GF compared with broilers fed the 0% or 0.75% Gly diets. Broilers fed the 1.47% Glu diet had decreased ADG and GF (*P* < 0.04) compared with broilers fed the PC diet. Broilers fed the 0% Gly diet had decreased ADG and GF (*P* < 0.01) compared with broilers fed the PC diet. Broilers fed the 0.750% Gly diet had similar ADFI and GF compared with broilers fed the PC; however, ADG tended to decrease (*P* = 0.11) compared with broilers fed the PC. A single slope, breakpoint analysis of GF estimated the Gly + Ser requirement to be 2.239% in a diet with 0.25% L-Lys•HCl (*P* = 0.37).

When the GF data from all 3 Exp. were combined (Figure 4.1), a single slope, breakpoint analysis estimated the Gly + Ser requirement to be 2.078% (P < 0.03).

Table 4.2. Glycine requirement of broiler chicks as assessed by growth performance in Experiment 1[1]

Treatment	ADG, g	ADFI, g	GF, g/g
1) Positive control (PC)	30.86	40.24	0.767
2) 0% crystalline Gly	31.12	41.37	0.752[a]
3) 0.125% crystalline Gly	31.83	42.12	0.756
4) 0.250% crystalline Gly	30.15	39.42	0.765
5) 0.375% crystalline Gly	31.84	40.98	0.776
6) 0.500% crystalline Gly	31.14	40.35	0.772
7) 0.625% crystalline Gly	31.45	41.75	0.753
8) 0.750% crystalline Gly	31.98	40.66[c]	0.786[a,b]
9) 1.470% crystalline Glu	32.22	42.57[a]	0.751[a]
SEM	0.677	0.822	0.006
P-value	0.508	0.202	0.001
Linear[2]	0.439	0.660	0.001
Quadratic	0.494	0.359	0.664

[1] Data are means of 7 replications with 6 chicks per replicate.
[2] Linear and quadratic contrasts were run on diets 2 to 8.
[a] Significantly different (*P* < 0.10) from PC.
[b] Significantly different (*P* < 0.01) from diet 9 with 1.47% L-Glu.
[c] Tended to be significantly different (*P* < 0.12) from diet 9 with 1.47% L-Glu.

Table 4.3. Glycine requirement of broiler chicks as assessed by growth performance in Experiment 2[1]

Treatment	ADG, g	ADFI, g	GF[2], g/g
1) Positive control (PC)	37.62	46.42	0.811
2) 0% crystalline Gly	37.21	49.45[a]	0.753[a,c]
3) 0.125% crystalline Gly	37.92	49.19	0.771
4) 0.250% crystalline Gly	36.96	47.37	0.782
5) 0.375% crystalline Gly	38.03	48.01	0.792
6) 0.500% crystalline Gly	38.09	49.12	0.776
7) 0.625% crystalline Gly	38.18	47.47	0.794
8) 0.750% crystalline Gly	36.85	46.07[c]	0.800[b,c]
9) 1.470% crystalline Glu	37.50	48.35	0.776[a]
SEM	0.704	0.967	0.005
P-value	0.846	0.144	0.001
Linear[3]	0.881	0.025	0.001
Quadratic	0.284	0.656	0.075

[1] Data are means of 7 replications with 6 chicks per replicate.
[2] Based on the GF means, a single slope, one breakpoint broken-line analysis estimated that the Gly + Ser requirement to be 2.085% total Gly + Ser or 0.309% crystalline Gly addition (P = 0.044 and SEM = 0.099).
[3] Linear and quadratic contrasts were run on diets 2 to 8.
[a] Significantly different ($P < 0.10$) from PC.
[b] Tended to be significantly different ($P < 0.12$) from PC.
[c] Significantly different (P < 0.10) from diet 9 with 1.47% L-Glu.

Table 4.4. Glycine requirement of broiler chicks as assessed by growth performance in Experiment 3[1]

Treatment	ADG, g	ADFI, g	GF, g/g
1) Positive control (PC)	34.67	44.29	0.783
2) 0% crystalline Gly	32.48[a,c]	42.98	0.756[a,c]
3) 0.125% crystalline Gly	32.52	41.61	0.782
4) 0.250% crystalline Gly	33.51	43.31	0.774
5) 0.375% crystalline Gly	32.99	42.78	0.772
6) 0.500% crystalline Gly	33.04	42.83	0.782
7) 0.625% crystalline Gly	33.27	42.50	0.782
8) 0.750% crystalline Gly	33.35[b]	43.08	0.774
9) 1.470% crystalline Glu	32.91[a]	43.35	0.759[a]
SEM	0.576	0.667	0.007
P-value	0.266	0.330	0.074
Linear[2]	0.239	0.649	0.106
Quadratic	0.635	0.902	0.126

[1] Data are means of 7 replications with 6 chicks per replicate.
[2] Linear and quadratic contrasts were run on diets 2 to 8.
[a] Significantly different (P < 0.10) from PC.
[b] Tended to be significantly different (P < 0.11) from PC.
[c] Significantly different (P < 0.10) from diet 9 with 1.47% L-Glu.

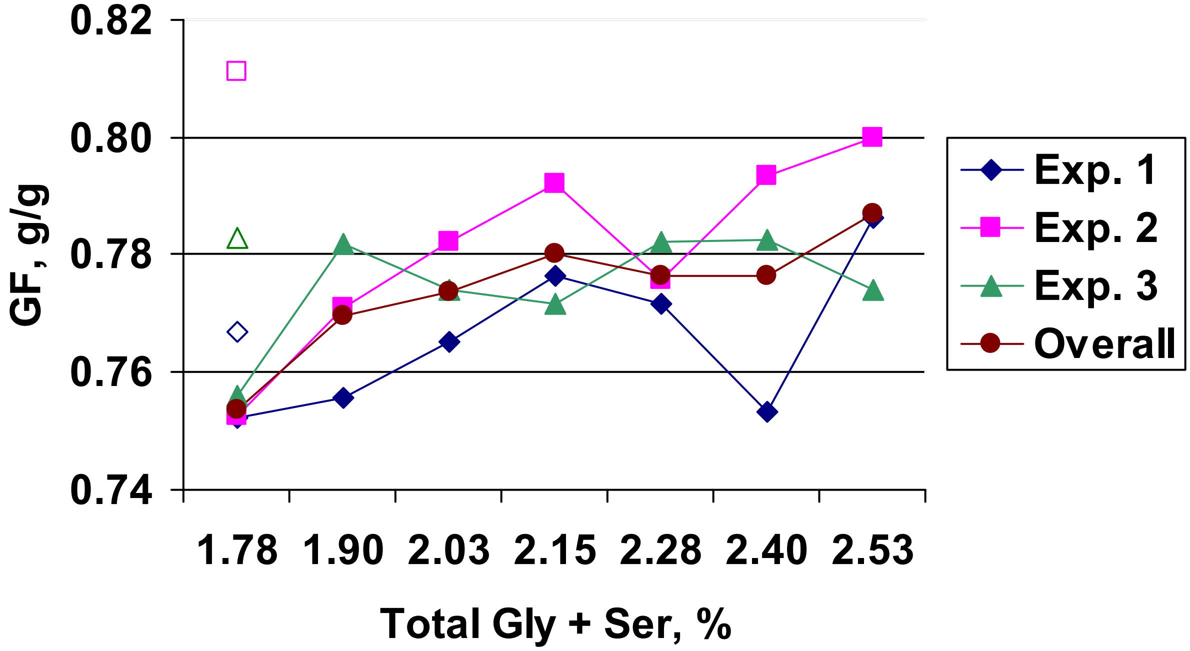

Figure 4.1. Gain:feed for broilers to determine the glycine requirement for Experiments 1, 2, and 3.

Table 4.5. Overall broken-line estimates of the glycine requirement for Experiments 1, 2, and 3[1]

Response	One-slope estimate	Two-slope estimate
GF	2.078	1.924
SEM	0.076	0.060
P-value	0.021	0.038

[1] Broken-line was initially estimated at 0.375% crystalline Gly addition (2.151% total Gly + Ser).

DISCUSSION

The objective of these Exp. was to determine the Gly requirement for broilers fed a low CP, C-SBM diet supplemented with 0.25% L-Lys•HCl, a level that previously has been shown to provide optimal growth performance for Ross x Ross 708 broilers (See chapter 3). In all Exp., the incremental crystalline Gly addition had no linear or quadratic effect on ADG or ADFI except in Exp. 2, where ADFI decreased linearly with the crystalline Gly additions. In Exp. 1, broilers fed the 0.625% Gly diet had similar GF to broilers fed the 0% Gly diet. We have no explanation for this response, but the overall GF response was linear. The analyzed value for the total Gly + Ser in the 0.625% Gly diet was lower than the formulated value, but still greater than the 0.500% Gly diet. In Exp. 2, the reduced GF of the broilers fed the 0.500% Gly diet was due to the increased ADFI. In all Exp., the incremental crystalline Gly addition had an increasing linear response on GF. Based on GF, there is a consistent response to crystalline Gly in a low CP, AA-supplemented diet.

In Exp. 1, a requirement estimate could not be made with either a single or two-slope broken-line analysis. In Exp. 2 and 3, a single slope, breakpoint analysis of GF estimated the Gly + Ser requirement to be 2.085% and 2.239%, respectively, in a diet with 0.25% L-Lys•HCl. When the GF data from all Exp. were combined, a single slope, breakpoint analysis estimated the total Gly + Ser requirement to be 2.078%.

In all Exp., broilers fed the 0% Gly diet had decreased GF compared with broilers fed the PC diet. This decreased GF indicates that 1.776% total Gly + Ser is below the requirement for total Gly + Ser.

Diet 9 was formulated to be isonitrogenous to the 0.75% Gly by supplementing 1.47% L-Glu. This diet was included in order to evaluate whether the broilers were responding to Gly or to NSN. In Exp. 1 and 2, ADFI tended to be significantly higher in broilers fed the diet containing 1.47% L-Glu compared with broilers fed the 0.750% Gly diet. In Exp. 1 and 2, broilers fed the 1.47% L-Glu diet had significantly decreased GF compared with broilers fed the 0.750% Gly diet, and in Exp. 3 the broilers fed the 1.47% L-Glu diet had decreased GF compared with broilers fed the 0.750% Gly diet although the difference was not significant. These results suggest that the improvement in GF is a response to Gly and not a result of added nitrogen from Glu. Other researchers have reported on the lack of improvement in broiler performance with supplementation of varying levels of L-Glu to low CP diets (Fancher and Jensen, 1989a,b; Pinchasov et al., 1990; Kerr and Kidd, 1999; and Bregendahl et al., 2002).

According to NRC, the total Gly + Ser required for 0- to 3-wk old broilers is 1.25%; however, our data indicates that the Gly + Ser requirement might be higher in a low CP diet. Corzo et al. (2004) also indicates that the Gly + Ser requirement suggested by NRC may be inadequate for broiler chicks fed low CP diets. They reported that the Gly + Ser requirement in 7-to 20-d old broilers was 1.80% in a low CP diet. Our results are based on a 0-d post-hatching broiler through 18-d; whereas, Corzo et al. (2004) is based on a 7- to 20-d broiler. Previous researchers have reported that the Gly + Ser requirement might be higher in post-hatching birds (0- to 5- or 9-d); whereas, a 6- or 10- to 15-d broiler would have a lower Gly + Ser requirement (Coon et al., 1974 and Ngo et al., 1976). Dean et al. (2006) reported that the minimum level of total Gly + Ser in a low CP diet should be 2.32% to achieve optimal performance of 0- to 17-d broilers, but this diet was a 16% CP, which is a very low CP diet.

Based on these three Exp., the Gly requirement in a low CP, C-SBM diet supplemented with 0.25% L-Lys•HCl is 2.078% total Gly + Ser. Our results indicate that a low CP, C-SBM diet which contains 0.25% L-Lys•HCl and supplemental DL-Met and L-Thr with the addition of crystalline Gly at 0.302% is a practical diet that can be fed to broilers to obtain optimal growth performance.

CHAPTER 5

SUMMARY AND CONCLUSIONS

This research was conducted to determine the optimal level of crystalline AA supplementation in low CP diets and the Gly requirement in low CP, AA-supplemented diets for broilers.

Three Exp. were conducted to determine the optimal level of L-Lys•HCl supplementation in a C-SBM diet that elicited optimal growth performance of broilers. Feeding a C-SBM based diet that is supplemented with 0.25% L-Lys•HCl and crystalline DL-Met, Thr, and Gly results in no negative effects on growth performance of broilers.

Two Exp. were conducted to determine the fifth, sixth, and seventh limiting AA in low CP diets for broilers. In a C-SBM diet fed to 0- to 18- d broilers, Val and Arg are the next limiting and the seventh limiting AA is Ile after Met, Lys, Thr, and Gly.

Three Exp. were conducted to determine the total Gly + Ser requirement in a low CP, C-SBM diet containing 0.25% L-Lys•HCl. These Exp. indicate that the Gly + Ser requirement is 2.078% total Gly + Ser. A C-SBM diet supplemented with 0.25% L-Lys•HCl, 0.302% crystalline Gly, crystalline DL-Met, and Thr can be fed to broilers to achieve optimal growth performance.

In conclusion, when feeding broilers low CP, C-SBM diets, crystalline Gly needs to be supplemented in the diet to achieve optimal growth performance. The reduction in growth performance in broilers fed low CP diets seems to be improved with crystalline Gly addition to the diet; therefore, there is a higher requirement for Gly in low CP diets. Further research is needed to determine specifically how the broilers are utilizing Gly in these low CP diets, and to determine the effects of additions of crystalline Gly to low CP diets fed to broilers at other stages of growth.

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APPENDIX:

LIST OF ABBREVIATIONS

Item	Abbreviation
Amino acid(s)	AA
Analysis of variance	ANOVA
Average daily feed intake	ADFI
Average daily gain	ADG
Corn	C
Crude protein	CP
Essential amino acid(s)	EAA
Experiment	Exp.
Feed conversion ratio	FCR
Gain:feed	GF
General linear model	GLM
Negative control	NC
Non-essential amino acid(s)	NEAA
Non-specific nitrogen	NSN
Positive control	PC
Soybean meal	SBM
Total sulfur amino acid(s)	TSAA

VITA

April Marie Waguespack, daughter of Galyn and Michele Waguespack, was born in Baton Rouge, Louisiana, on April 25, 1983. April is the youngest of two children. She attended a college preparatory high school at St. Joseph's Academy in Baton Rouge, Louisiana. After graduating in 2001, she was accepted to Louisiana State University in Baton Rouge, Louisiana. In December of 2005, she completed a Bachelor of Science degree with a concentration in animal sciences. In January of 2006, she began her pursuit of a Master of Science degree with a concentration in animal sciences at Louisiana State University.