

Effects of lysine biomass supplementation on growth performance and clinical indicators in broiler chickens

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ABSTRACT Production of crystalline amino acids (AA) through microbial fermentation concomitantly provides an AA-enriched biomass that may serve as a cost-effective supplement for broiler chickens. We investigated the effects of feeding a fermentation biomass product containing approximately 62% Lys on growth performance, organ growth, and clinical outcomes of broilers. Beginning at 2 d post-hatch, a total of 360 Ross 308 chicks were randomly assigned to 1 of 5 treatments provided to 12 replicate cages of 6 birds. Practical corn-soybean meal-based dietary treatments included: negative control (NC; no supplementation of L-Lys, 1.01 and 0.86% standardized ileal digestible Lys in starter and grower phases, respectively), NC + 0.23% L-Lys HCl (positive control; PC), and NC supplemented with 0.30, 0.90, or 1.50% Lys biomass (LB) in both phases. Feed and water were provided ad libitum throughout the study. Individual bird and feeder weights were recorded on study day 0, 10, 21, and 35. At study conclusion, birds from each treatment were

randomly selected to collect blood and tissue samples. The PC and 0.30% LB diets elicited similar overall (day 0–35) body weight gain and birds were heavier ($P < 0.001$) than the NC and other LB treatments. The PC, 0.30% LB, and 0.90% LB groups had better ($P < 0.001$) overall feed conversion ratio than NC. Some LB-supplemented treatments elicited increased ($P < 0.001$) relative spleen and ileum weight compared with NC and PC. Heterophils were increased ($P < 0.001$) in LB treatments compared with PC and NC. Lymphocytes were decreased ($P < 0.001$) in LB treatments compared with NC, and 1.50% LB was similar to PC. This resulted in an increased ($P < 0.001$) heterophil-to-lymphocyte ratio in some LB treatments, which may have resulted from general AA supplementation or the LB product. Collectively, these results suggest that addition of up to 0.30% LB restored growth performance when added to a Lys-deficient practical diet and elicited results identical to the Lys-adequate PC diet with no negative clinical effects.

Key words: lysine, broiler, biomass, performance, clinical chemistry

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INTRODUCTION

Protein supplements constitute a relatively expensive component of poultry feeds, second only to the energy yielding raw materials, which require nutritionists to be attentive about the protein and energy levels of the feed. Meeting the bird's amino acid (AA) requirements contributes considerably to the overall cost of the feed. Availability of synthetic and crystalline AA sources allows nutritionists to reduce the inclusion of intact

protein sources (i.e., soybean meal and animal by-products) while meeting the AA needs of the bird and optimizing growth performance (Lipstein et al., 1975; Parr and Summers, 1991; Kidd et al., 2002). In addition, satisfying AA requirements while minimizing dietary CP also has positive implications toward environmental stewardship (Namroud et al., 2010; Hernández et al., 2012). Commercial availability of supplemental AA, and the resulting improvements in growth, health, and productivity from their supplementation, largely determines the degree to which practical nutritionists can further reduce dietary CP levels (Si et al., 2004; Dean et al., 2006; Namroud et al., 2010). Currently, the first 5 limiting AA (i.e., Met, Lys, Thr, Val, and Ile) are commercially available and routinely added to poultry diets (Kidd et al., 1997; Si et al., 2004; Waguespack et al., 2009). Although Met is typically the first limiting

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AA in poultry feeds, Lys is the most important AA for the ideal protein concept in feed formulation as it is used as the reference AA (Emmert and Baker, 1997). Given the importance of Lys, there exists potential for market introduction of other Lys containing products that may offer nutritional, environmental, and economic benefits to producers.

Feed-grade quality Lys can be produced by bacterial fermentation of carbohydrate sources through genetic enhancement of bacterial species, including *Micrococcus glutamicus* (Kinoshita et al., 1958) and *Corynebacterium glutamicum* (Nakayama et al., 1961; Kelle et al., 2005). As such, *C. glutamicum* is the source of nearly all crystalline L-Lys HCl used commercially today as derived through fermentation processes (Kelle et al., 2005). Rather than extracting the produced Lys and discarding the fermentation broth with inactivated *C. glutamicum* cells and excess nutrients, the entire product—called a biomass—may have nutritive and economic potential. This biomass contains relatively high concentrations of L-Lys, in addition to other nutrients, such as phosphorous and additional AA. In an effort to utilize these nutrients and reduce waste emissions, the Lys biomass (LB) has been proposed as an alternative source of dietary Lys for livestock species.

Both the concentration and bioavailability of Lys in the final biomass product are dependent on processing methods applied to the fermentation broth (Höfler et al., 1998; Kelle et al., 2005). As such, LB may contain a slightly lower concentration of Lys compared with crystalline L-Lys HCl (78.8% L-Lys), but it also provides additional AA and other nutrients that must be considered when assigning nutritional value (Kelle et al., 2005). To this end, the objective of the current study was to investigate whether graded dietary concentrations of a novel LB product would be efficacious and safe when fed to broiler chickens. We hypothesized that Lys supplementation via a LB product would restore growth of broilers as induced by a mild dietary Lys deficiency in practical diets.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to initiation of the experiment.

Bird Husbandry and Experimental Design

Day-old Ross 308 chicks were obtained from a commercial hatchery (Hoover's Hatchery, Rudd, IA) and transported to the University of Illinois Edward R. Madigan Laboratory. Chicks were placed in thermostatically controlled cages (model SB5T; Alternative Design Manufacturing, Siloam Springs, AR) with raised wire flooring in an isolated, environmentally controlled room with continuous lighting. Chicks were fasted overnight in the cages with access only to water to permit study commencement at 2 d post-hatch. Standard

corn-soybean meal-based diets (Table 1) were formulated to meet or exceed requirements of broilers (NRC, 1994) for starter and grower phases with the exception of the negative control (NC) diet being slightly deficient in Lys. Feed and water were provided ad libitum throughout the study.

A total of 360 chicks at 2 d post-hatch were weighed, selected, wing-banded, and assigned to 1 of 5 dietary treatment groups with a total of 6 birds allotted to each of 12 replicate cages (99 cm × 34 cm) per treatment. The average initial group weights and weight distributions were similar across treatments and treatments were arranged in a randomized complete block design.

Test Article and Dietary Treatments

The test article used in this experiment was a Lys-enriched fermentation biomass product (Biolsy77; Evonik Operations GmbH, Nutrition & Care, Hanau-Wolfgang, Germany), herein referred to as LB, that was analyzed to contain 62.23% Lys. Further analytical details of this ingredient can be found in Table 2. A 1.50% formulation space was reserved to permit supplementation of either the LB or L-Lys HCl in combination with fine-ground corn to produce a premix that was used in the following dietary treatment groups: NC (basal diet without L-Lys supplementation and containing 1.01 and 0.86% standardized ileal digestible [SID] Lys in the starter and grower phases, respectively), NC + 0.23% L-Lys HCl (positive control [PC]; containing 1.19 and 1.04% SID Lys in the starter and grower phases, respectively), NC + 0.30% LB (0.30% LB; containing 1.20 and 1.05% SID Lys in the starter and grower phases, respectively), NC + 0.90% LB (0.90% LB; containing 1.57 and 1.42% SID Lys in the starter and grower

Table 1. Ingredient composition (%) of the basal diet for each feeding phase.

Ingredient	Starter (day 0–21)	Grower (day 22–35)
Corn	58.56	64.61
Soybean meal	33.43	27.06
Soybean oil	2.42	3.37
Dicalcium phosphate	1.91	1.49
Limestone	0.85	0.74
Salt	0.38	0.31
Sodium bicarbonate	0.00	0.10
Poultry vitamin premix ¹	0.20	0.20
Poultry mineral premix ²	0.15	0.15
Choline chloride, 60%	0.12	0.10
DL-Met	0.32	0.25
L-Thr	0.09	0.07
L-Val	0.06	0.03
L-Ile	0.02	0.02
Test article premix ³	1.50	1.50

¹Provided per kg of complete diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL- α -tocopheryl acetate, 11 IU; vitamin B12, 0.01 mg; riboflavin, 4.41 mg; D-Ca-pantothenate, 10 mg; niacin, 22 mg; and menadione sodium bisulfite complex, 2.33 mg.

²Provided per kg of complete diet: Mn, 75 mg from MnO; Fe, 75 mg from FeSO₄·7H₂O; Zn, 75 mg from ZnO; Cu, 5 mg from CuSO₄·5H₂O; I, 0.75 mg from ethylene diamine dihydroiodide; and Se, 0.1 mg from Na₂SeO₃.

³A total of 1.5% of the basal formulation was reserved for the combined addition of test articles plus fine-ground corn, as denoted by 'Test article premix' in the ingredient list.

Table 2. Analyzed nutritional content (% , as-fed basis) of the lysine biomass.¹

Item	Value
DM	98.71
Moisture	1.29
CP	75.40
Arg	0.62
Gly	0.31
His	0.11
Ile	0.26
Leu	0.53
Lys	62.23
Met	0.08
Phe	0.18
Thr	0.25
Val	0.34
Crude fiber	<0.20
Crude fat	1.50
Crude ash	1.20
Calcium	0.02
Phosphorous	0.13
Magnesium	0.04
Sodium	0.10
Potassium	0.30

Abbreviation: AOAC, Association of Official Analytical Chemists.

¹Analyses were performed using the following methods: DM, calculated as 100% – moisture; moisture, AOAC 2001.12; CP, AOAC 990.03; amino acids, AOAC 994.12; crude fiber, ANKOM Technology method; crude fat, modified AOAC 954.02; crude ash, AOAC 942.05; minerals, modified AOAC 985.01.

phases, respectively), and NC + 1.50% LB (1.50% LB; containing 1.94 and 1.79% SID Lys in the starter and grower phases, respectively). The inclusion rates of the LB product at 0.30, 0.90, and 1.50% of the diet were designed to provide 1, 3, and 5 times the purported Lys requirement of the bird, respectively, and apart from Lys, diets were not balanced for any other nutrient or energy in accounting for addition of the test article premix. Based on the analyzed product purity, 0.23% L-Lys HCl was assumed to provide an equivalent concentration of Lys compared with 0.30% LB. Starter phase diets were provided as crumbles from study day 0 to 10 and pellets from study day 10 to 21, while grower phase diets were provided as pellets from study day 21 to 35.

Growth Performance Data and Sample Collection

Birds and feeders were weighed on study day 0, 10, 21, and 35 for calculation of average body weight gain, average feed intake, and feed conversion ratio (FCR; g feed intake:g body weight gain) to assess growth performance. Mortality and culls were monitored daily and used to adjust feed intake and FCR. On study day 35, all birds were euthanized using carbon dioxide gas and 1 bird per cage (representing 16.7% of all birds per treatment) was randomly selected for sample collection. Blood was collected by cardiac puncture into evacuated tubes. Each sample (approximately 2 mL per bird) was submitted to the

Veterinary Clinical Pathology Laboratory at the University of Illinois at Urbana-Champaign for quantifying hematological outcomes. The following clinical hematology parameters were quantified by performing manual differentials: hematocrit, total protein, heterophils, lymphocytes, mono/azuro granules, eosinophils, and basophils. Total white blood cell counts were not captured due to the unreliability of automated cell counting equipment with avian blood cells, and manual cell counts were not possible with the high number of blood samples collected. Therefore, white blood cell differential profiles were determined by manually counting 100 cells on a stained blood smear. The hematocrit and total protein values were obtained by reading the packed red cells and plasma, respectively, on a refractometer. The following clinical chemistry parameters were also quantified (AU680 analyzer; Beckman Coulter, Inc., Brea, CA): albumin (modified Doumas and Rodkey procedures), calcium (calcium o-cresolphthalein complexone procedure), phosphorous (modified Daly and Ertingshausen procedures), glucose, aspartate aminotransferase (AST; modified International Federation of Clinical Chemistry method), creatine phosphokinase (CPK; modified International Federation of Clinical Chemistry method), and glutamate dehydrogenase (Randox, Cruclin, UK). Following blood collection, the same randomly selected bird per cage was dissected to permit collection and measurement of organs, including liver, spleen, proventriculus, gizzard, pancreas, ileum, ceca, and heart. Absolute weights were recorded for each organ and the relative weight was calculated using the final body weight of the individual bird.

Treatment Blinding and Analysis of Experimental Diets

All personnel at the University of Illinois were blinded to dietary treatments until after the live-animal phase had ended. As such, all feed and test material used during this experiment were prepared off-site (Kansas State University, Manhattan, KS) and labeled using a non-descriptive color-coding system. A homogenous and representative sample of each basal and experimental diet was collected and used for analysis of nutrient composition. All analyses were performed by AMINO-Lab (Evonik Product Quality & Regulatory Affairs, Hanau, Germany; Tables 3 and 4) with DM, CP, and apparent metabolizable energy analyzed using near infrared spectroscopy; DM was standardized at 88% for expression of analyzed nutrients. Other proximate compositional components were analyzed according to standardized and validated methods including ether extract (i.e., crude fat; Association of Official Analytical Chemists [AOAC] method 954.02), ash (AOAC method 942.05), crude fiber (ANKOM Technology method), CP (AOAC method 990.03), sugar (AOAC method 945.66, Total Reducing Sugars), and starch (AOAC new method 2014.10 [4.7.07]). The AA analysis was

Table 3. Analyzed nutritional content (% , as-fed basis) of starter phase diets.¹

Item	Dietary treatment				
	NC	PC	0.30% LB	0.90% LB	1.50% LB
DM	88.60	88.77	88.38	87.96	88.41
CP	20.99	21.17	21.76	21.92	22.45
Total AA	19.8	20.38	20.55	20.58	21.23
Arg	1.38	1.40	1.42	1.40	1.42
Gly	0.85	0.86	0.87	0.86	0.87
His	0.54	0.55	0.56	0.55	0.55
Ile	0.90	0.93	0.92	0.91	0.92
Leu	1.77	1.78	1.76	1.77	1.77
Lys	1.12	1.33	1.40	1.68	2.13
Met	0.59	0.63	0.64	0.63	0.61
Phe	1.02	1.04	1.04	1.04	1.04
Thr	0.87	0.90	0.89	0.87	0.90
Val	1.02	1.07	1.06	1.03	1.04
Crude fiber	2.6	2.5	2.5	2.5	2.3
ADF	4.0	4.2	3.9	3.8	3.5
NDF	9.5	9.4	9.1	9.8	8.6
Crude fat	4.8	4.8	4.6	4.9	5.2
Crude ash	5.5	5.3	5.4	5.5	5.4
Starch	39.8	40.5	39.4	37.6	38.2
Sugars	4.0	3.9	4.0	4.1	4.6
Phosphorus	0.60	0.54	0.60	0.61	0.59

Abbreviations: AA, amino acid; ADF, acid detergent fiber; LB, Lys biomass; NC, negative control; NDF, neutral detergent fiber; PC, positive control.

¹Apparent metabolizable energy (kcal/kg) was analyzed using near infrared spectroscopy and was as follows: NC, 2881; PC, 2907; 0.30% LB, 2866; 0.90% LB, 2820; 1.50% LB, 2899. Standardized ileal digestible Lys (%) was calculated as follows: NC, 1.01; PC, 1.19; 0.30% LB, 1.20; 0.90% LB, 1.57; 1.50% LB, 1.94.

conducted using an AA analyzer (Biochrom 30+, Cambridge, UK).

Table 4. Analyzed nutritional content (% , as-fed basis) of grower phase diets.¹

Item	Dietary treatment				
	NC	PC	0.30% LB	0.90% LB	1.50% LB
DM	88.80	88.32	88.45	88.89	89.18
CP	19.05	19.05	18.99	19.44	19.72
Total AA	18.00	18.16	18.17	18.78	18.74
Arg	1.25	1.22	1.25	1.26	1.24
Gly	0.77	0.77	0.77	0.78	0.76
His	0.49	0.49	0.49	0.50	0.49
Ile	0.80	0.80	0.80	0.82	0.80
Leu	1.65	1.64	1.61	1.64	1.60
Lys	1.01	1.18	1.20	1.60	1.95
Met	0.51	0.54	0.54	0.55	0.55
Phe	0.93	0.92	0.93	0.95	0.91
Thr	0.78	0.77	0.79	0.81	0.78
Val	0.89	0.89	0.89	0.91	0.89
Crude fiber	2.4	2.2	2.2	2.1	2.4
ADF	4.0	3.5	3.6	3.5	3.7
NDF	9.2	8.8	8.7	8.4	9.2
Crude fat	5.7	5.7	5.8	5.7	5.9
Crude ash	5.0	5.1	5.2	4.8	4.6
Starch	43.3	42.4	41.7	43.2	42.5
Sugars	3.4	3.4	3.8	3.7	3.7
Phosphorus	0.51	0.52	0.54	0.48	0.49

Abbreviations: AA, amino acid; ADF, acid detergent fiber; LB, Lys biomass; NC, negative control; NDF, neutral detergent fiber; PC, positive control.

¹Apparent metabolizable energy (kcal/kg) was analyzed using near infrared spectroscopy and was as follows: NC, 2985; PC, 2959; 0.30% LB, 2958; 0.90% LB, 2993; 1.50% LB, 2997. Standardized ileal digestible Lys (%) was calculated as follows: NC, 0.86; PC, 1.04; 0.30% LB, 1.05; 0.90% LB, 1.42; 1.50% LB, 1.79.

Statistical Analysis

Each cage, or the single bird sampled per cage for organ and blood parameters, served as the experimental unit for all outcomes with 12 replicate cages allotted at the start of the study for each of 5 dietary treatments, as arranged in a randomized complete block design. Growth performance data were corrected for mortality and all outcomes were subjected to an ANOVA using the Mixed procedure of SAS (version 9.4; SAS Institute, Cary, NC). A 1-way ANOVA was used to determine whether the model was significant, and when appropriate, means separation of the 5 treatments was conducted. Least-square means and SEM estimates were derived from this 1-way ANOVA. Outliers were identified as having an absolute studentized residual value of 3 or greater and were removed. Two treatments were declared significantly different if the *P*-value for that comparison was less than or equal to 0.05. For outcomes where there were one or more missing values, the highest SEM was reported as the pooled SEM for that outcome.

RESULTS

Growth Performance

Average body weight gain (Table 5) for NC birds was lower ($P < 0.001$) at all time points (except day 0–10) when compared with PC and 0.30% LB treatments, and PC and 0.30% LB groups had similar weight gain at all time points. Additionally, birds receiving 0.90% LB had similar overall (day 0–35) weight gain compared with NC, while 1.50% LB had the overall lowest ($P < 0.001$) weight gain compared with all other treatments. Birds assigned to the NC group had lower ($P < 0.001$) overall feed intake when compared with PC-fed birds, while PC and 0.30% LB groups had similar feed intakes at all time points. Birds fed 0.90% LB exhibited similar overall feed intake when compared with NC and 1.50% LB groups, while 1.50% LB-fed birds had lower ($P < 0.001$) feed intake than NC birds. Overall FCR for PC-fed birds was lower (i.e., better; $P < 0.001$) than for NC-fed birds, while birds fed 0.30% LB had similar, but numerically lower, overall FCR compared with the PC group. Finally, NC and 1.50% LB groups had the highest (i.e., worst; $P < 0.001$) overall FCR, whereas 0.90% LB-fed birds were more efficient with an FCR response equivalent to the PC group. No difference in mortality was observed between treatment groups.

Organ Weights

The PC and NC treatments elicited similar absolute and relative organ weights (Table 6) of the liver, spleen, proventriculus, and ileum, whereas the absolute gizzard weight of PC birds was greater ($P < 0.01$) than that of NC birds at study conclusion. No treatment differences in absolute or relative weights for ceca or heart were observed. Birds fed 0.30% LB had increased ($P < 0.05$)

Table 5. Growth performance of broilers fed experimental diets of differing Lys concentrations.¹

Item	Dietary treatments					Pooled SEM	Model <i>P</i> -value
	NC	PC	0.30% LB	0.90% LB	1.50% LB		
Body weight, g/chick							
Day 0	41	41	41	41	41	1.3	1.000
Day 10	297 ^{a,b}	310 ^a	310 ^a	277 ^{b,c}	270 ^c	8.5	0.002
Day 21	980 ^b	1,089 ^a	1,088 ^a	932 ^c	807 ^d	14.0	<0.001
Day 35	2,042 ^b	2,333 ^a	2,340 ^a	2,119 ^b	1,874 ^c	37.3	<0.001
Body weight gain, g/chick							
Day 0–10	256 ^{a,b}	269 ^a	270 ^a	237 ^{b,c}	229 ^c	7.5	<0.001
Day 10–21	683 ^b	774 ^a	763 ^a	652 ^c	536 ^d	10.7	<0.001
Day 21–35	1,063 ^b	1,220 ^a	1,260 ^a	1,187 ^a	1,061 ^b	31.0	<0.001
Day 0–35	2,001 ^b	2,291 ^a	2,299 ^a	2,078 ^b	1,834 ^c	37.2	<0.001
Feed intake, ² g/chick							
Day 0–10	303 ^a	309 ^a	298 ^{a,b}	283 ^{b,c}	278 ^c	7.3	0.010
Day 10–21	940 ^{b,c}	995 ^a	954 ^{a,b}	892 ^c	811 ^d	19.5	<0.001
Day 21–35	2,029 ^{a,b}	2,154 ^a	2,106 ^a	1,943 ^{b,c}	1,847 ^c	46.6	<0.001
Day 0–35	3,147 ^{b,c}	3,351 ^a	3,192 ^{a,b}	2,973 ^{c,d}	2,813 ^d	68.9	<0.001
Feed conversion ratio, ² g/g							
Day 0–10	1.18 ^b	1.11 ^c	1.11 ^c	1.20 ^{a,b}	1.23 ^a	0.013	<0.001
Day 10–21	1.38 ^b	1.32 ^b	1.25 ^c	1.37 ^b	1.51 ^a	0.025	<0.001
Day 21–35	1.91 ^a	1.77 ^b	1.69 ^b	1.64 ^b	1.75 ^b	0.046	0.001
Day 0–35	1.57 ^a	1.41 ^{b,c}	1.39 ^c	1.46 ^b	1.56 ^a	0.025	<0.001

^{a–d}Means without a common superscript letter differ within a row ($P < 0.05$).

Abbreviations: LB, Lys biomass; NC, negative control; PC, positive control.

¹Values are least square means derived at 2 to 37 d post-hatch (study day 0–35) from between 9 and 12 replicate cages allotted with 6 chicks at study initiation.

²Values have been corrected for mortality.

absolute weights of liver, spleen, gizzard, pancreas, and ileum and increased ($P < 0.001$) relative weights of spleen and ileum when compared with the NC group. The 0.30% LB group elicited weights that were similar to the PC group for all organs except for the absolute and relative spleen and ileum weights, where 0.30% LB birds had higher ($P < 0.001$) weights than PC birds. The 1.50% LB diet elicited increased ($P < 0.05$) absolute and relative weights for all organs, except ceca and heart, when compared with the NC diet. Birds receiving 0.90% LB had similar relative weights as the NC and PC groups for all organs except proventriculus and ileum,

where 0.90% LB-fed birds had increased ($P < 0.05$) weights.

Blood Parameters

For the clinical chemistry outcomes (Table 7), the PC and NC treatments elicited similar responses for all parameters except calcium, where levels were higher ($P < 0.001$) in PC-fed birds. There were no differences observed among treatments for albumin, phosphorous, or glutamate dehydrogenase. All LB-supplemented treatments were similar to PC for calcium, and 1.50%

Table 6. Absolute and relative organ weights of broilers fed experimental diets of differing Lys concentrations.¹

Item	Dietary treatments					Pooled SEM	Model <i>P</i> -value
	NC	PC	0.30% LB	0.90% LB	1.50% LB		
Absolute weight, g							
Liver	41.3 ^b	47.3 ^{a,b}	52.0 ^a	47.6 ^{a,b}	51.4 ^a	2.41	0.015
Spleen	1.5 ^b	1.6 ^b	2.3 ^a	1.7 ^b	2.6 ^a	0.18	<0.001
Proventriculus	7.8 ^b	7.9 ^b	9.3 ^{a,b}	9.7 ^a	9.7 ^a	0.64	<0.001
Gizzard	35.8 ^b	44.4 ^a	46.7 ^a	43.2 ^a	47.1 ^a	2.21	0.003
Pancreas	3.7 ^c	4.4 ^{b,c}	4.6 ^{a,b}	4.1 ^{a,b,c}	4.7 ^a	0.23	0.011
Ileum	15.2 ^b	16.2 ^b	25.4 ^a	22.1 ^a	21.8 ^a	1.72	<0.001
Ceca	13.8	16.8	14.2	15.8	15.9	1.55	0.569
Heart	13.0	13.3	14.7	13.7	14.3	0.60	0.257
Relative weight, g/kg BW							
Liver	20.0 ^b	20.3 ^b	22.2 ^{a,b}	21.6 ^{a,b}	23.9 ^a	0.83	0.007
Spleen	0.7 ^c	0.7 ^c	1.0 ^b	0.8 ^{b,c}	1.3 ^a	0.08	<0.001
Proventriculus	3.8 ^b	3.4 ^b	4.0 ^{a,b}	4.5 ^a	4.5 ^a	0.25	0.011
Gizzard	17.5 ^b	19.4 ^{a,b}	20.1 ^{a,b}	19.7 ^{a,b}	22.0 ^a	1.01	0.037
Pancreas	1.8 ^b	1.8 ^b	2.0 ^{a,b}	1.9 ^b	2.2 ^a	0.10	0.018
Ileum	7.3 ^b	6.9 ^b	10.8 ^a	10.0 ^a	10.1 ^a	0.68	<0.001
Ceca	6.7	7.3	6.1	7.2	7.3	0.66	0.565
Heart	6.6	6.0	6.3	6.3	6.6	0.27	0.388

^{a–c}Means without a common superscript letter differ within a row ($P < 0.05$).

Abbreviations: LB, Lys biomass; NC, negative control; PC, positive control.

¹Values are least square means derived from between 9 and 12 replicate cages allotted with 6 chicks at study initiation. Organ weights were collected from 1 chick per cage at study conclusion (37 d post-hatch or study day 35) immediately postmortem.

Table 7. Clinical chemistry of broilers fed experimental diets of differing Lys concentrations.¹

Item	Dietary treatments					Pooled SEM	Model <i>P</i> -value
	NC	PC	0.30% LB	0.90% LB	1.50% LB		
Albumin, g/dL	1.1	1.1	1.1	1.0	1.1	0.02	0.142
Calcium, mg/dL	10.4 ^c	11.0 ^{a,b}	11.2 ^a	10.9 ^{a,b}	10.6 ^{b,c}	0.12	<0.001
Phosphorous, mg/dL	7.0	7.2	6.7	7.0	6.9	0.13	0.081
Glucose, mg/dL	228.3 ^c	248.8 ^{b,c}	283.3 ^a	253.7 ^b	257.6 ^b	7.74	<0.001
Aspartate aminotransferase, U/L	264.2 ^b	293.1 ^b	331.6 ^{a,b}	420.9 ^a	430.2 ^a	41.91	0.011
Creatine phosphokinase, U/L	11,445 ^c	17,052 ^{b,c}	25,752 ^{a,b}	31,954 ^a	33,551 ^a	4,823.2	0.004
Glutamate dehydrogenase, U/L	0.8	1.3	1.0	1.5	1.3	0.23	0.208

^{a-c}Means without a common superscript letter differ within a row ($P < 0.05$).

Abbreviations: LB, Lys biomass; NC, negative control; PC, positive control.

¹Values are least square means derived from 9 to 12 replicate cages allotted with 6 chicks at study initiation. Blood was collected from 1 chick per cage at study conclusion (37 d post-hatch or study day 35) via cardiac puncture immediately postmortem.

LB was also similar to NC. The 0.30% LB group had the highest ($P < 0.001$) concentration of glucose compared with all other treatments, while 0.90 and 1.50% LB were similar to PC. AST levels were elevated in LB-supplemented groups, with levels for 0.90% LB and 1.50% LB treatments higher ($P < 0.05$) than both PC and NC. CPK levels were also elevated in the LB-supplemented groups, where they were all higher ($P < 0.05$) than NC, and 0.30% LB was similar to PC. For the hematology outcomes (Table 8), all reported values are expressed relative to total white blood cells. Birds receiving the PC diet had higher ($P < 0.05$) total protein compared with all other treatments, but PC and NC groups exhibited similar percentages of heterophils and lymphocytes. There were no differences observed among treatments for hematocrit, mono/azuro granules, eosinophils, or basophils. The percentage of heterophils was higher ($P < 0.001$) in all LB-supplemented treatments when compared with NC and PC treatments. The percentage of lymphocytes was higher ($P < 0.001$) in NC and PC birds when compared with all LB-supplemented treatments, except the PC group which was only numerically higher than the 1.50% LB group. This resulted in an increased ($P < 0.001$) heterophil-to-lymphocyte (H:L) ratio for 0.30% LB and 0.90% LB when compared with NC and PC.

DISCUSSION

Although not the first limiting AA in corn-soybean meal-based diets, Lys is arguably the most important

AA for broilers as it is used as the reference AA for the ideal protein concept in feed formulation (Emmert and Baker, 1997). Dietary addition of supplemental AA, such as Lys, allows nutritionists to meet the requirements to optimize broiler growth performance while also reducing the need for intact protein sources (Lipstein et al., 1975; Parr and Summers, 1991; Kidd et al., 2002). In the current study, we investigated whether graded dietary concentrations of a novel LB product would be efficacious and safe in supporting the growth and health of broiler chickens. Over the 35-day feeding period, we observed clear differences in growth performance between NC and PC groups, with few differences in blood or organ outcomes. Overall, the addition of 0.30% LB to the NC restored growth performance outcomes that matched or exceeded levels achieved by PC-fed birds. However, birds supplemented with LB at either 0.90 or 1.50% did not perform as well as the NC group, suggesting that a dietary inclusion threshold had been exceeded.

The experimental objective was achieved, with inclusion of 0.23% L-Lys HCl in the PC diet eliciting clear improvements in growth performance when compared with birds fed the unsupplemented NC diet. L-Lys HCl is a standard Lys supplement used in commercial poultry production with a guaranteed Lys purity of 78.8% and, as such, this source was used as a standard against which LB was tested. Birds fed the PC diet had higher overall weight gain when compared with NC, along with a significant improvement in the overall FCR. Moreover, differences between the PC and NC groups for organ

Table 8. Clinical hematology of broilers fed experimental diets of differing Lys concentrations.¹

Item	Dietary treatments					Pooled SEM	Model <i>P</i> -value
	NC	PC	0.30% LB	0.90% LB	1.50% LB		
Hematocrit, %	31.8	31.4	31.0	30.6	29.9	0.82	0.481
Total protein, g/dL	3.3 ^{b,c}	3.6 ^a	3.4 ^b	3.3 ^{b,c}	3.1 ^c	0.09	0.002
Heterophils, % of WBC	13.3 ^b	19.7 ^b	35.4 ^a	29.5 ^a	31.1 ^a	3.47	<0.001
Lymphocytes, % of WBC	81.8 ^a	75.5 ^{a,b}	57.0 ^c	61.1 ^c	64.8 ^{b,c}	4.02	<0.001
Heterophil-to-lymphocyte ratio	0.2 ^c	0.2 ^c	0.8 ^a	0.6 ^{a,b}	0.4 ^{b,c}	0.09	<0.001
Mono/azuro granules, % of WBC	2.1	3.6	2.1	3.2	3.0	0.55	0.182
Eosinophils, % of WBC	0.5	0.3	0.3	0.6	0.3	0.16	0.463
Basophils, % of WBC	2.3	3.1	3.0	4.7	3.7	0.98	0.418

^{a-c}Means without a common superscript letter differ within a row ($P < 0.05$).

Abbreviations: LB, Lys biomass; NC, negative control; PC, positive control; WBC, white blood cells.

¹Values are least square means derived from 9 to 12 replicate cages allotted with 6 chicks at study initiation. Blood was collected from 1 chick per cage at study conclusion (37 d post-hatch or study day 35) via cardiac puncture immediately postmortem.

weights and blood parameters were minimal, suggesting no adverse implications of supplementation with L-Lys HCl. This provided evidence that the NC diet was Lys-deficient, as intended, but still met or exceeded the [NRC \(1994\)](#) requirements for all other nutrients.

After validating the model with differentiation between the NC and PC diets, we evaluated the performance of birds fed graded supplementation levels of LB as compared with both control diets. Birds fed 0.30% LB grew as well as the PC birds and had numerically improved FCR, attributed by an overall feed intake lower than that of the PC. Although the FCR of the 0.90% LB treatment was also similar to the PC group, this similarity was a result of reduced feed intake and weight gain, not improvement. We found similar, but more exaggerated, results with the 1.50% inclusion rate of LB—it was evident (from body weight gain) that the inclusion of 1.50% LB slowed the growth rate of the broilers. For the diets supplemented with 1.50% LB, the total Lys content was analyzed at 2.13 and 1.95% for the starter and grower diets, respectively, which were nearly double the recommendations of the [NRC \(1994\)](#) for 1.10 and 1.00%, respectively. [Han and Baker \(1993\)](#) found numerical decreases in weight gain with little effect on feed efficiency (i.e., gain:feed or G:F) when the total Lys in the diet was 2.18% or higher for broiler chicks 8 to 22 d post-hatch. [Bouyeh \(2012\)](#) found decreased weight gain when feeding Ross 308 chicks diets with Lys at 40% more than the NRC recommendation (i.e., at 1.54 and 1.40% for starter and grower, respectively). Further research into the cause of this weight reduction found an antagonistic relationship between Lys and Arg, where excess dietary Lys elicited a deficiency of Arg, which therefore reduced weight gain ([Jones, 1964](#); [Jones et al., 1967](#)). More specifically, [Allen and Baker \(1972\)](#) found that feeding an excess of 1% dietary Lys increased the Arg requirement by 51%. By applying this same method to the 1.50% LB starter and grower diets (which achieved 1% excess Lys), the adjusted Arg requirements would have increased to 1.89 and 1.66%, respectively (calculated from the 1994 NRC requirement for Arg of 1.25 and 1.1%). Because the analyzed Arg content in the 1.50% LB diets was below the adjusted requirement, we can attribute the decreased growth performance of those birds to a deficiency of Arg. Although the 0.90% LB treatment supplied a lesser excess of 0.58 and 0.60% Lys for starter and grower diets, respectively, we still observed a reduction in weight gain, although to a lesser extent than 1.50% LB. As [Bouyeh \(2012\)](#) similarly reported decreased weight gain at only 40% above the [NRC \(1994\)](#) requirement for Lys, this provides evidence that the 0.90% LB treatment also provided Lys at excess high enough to elicit a deficiency of Arg, resulting in a reduction in weight gain. These findings suggest that, although supplementation of LB at 0.30% restored growth performance as well as 0.23% L-Lys-HCl, supplementation of LB at 0.90% or higher can result in growth depression attributed to Arg deficiency.

In addition to measuring the overall body weight gain, safety aspects can be ascribed to changes in absolute and relative weights of individual organs. Clearly, the principles of allometric growth state that absolute organ weights increase in proportion to body weight ([Brown et al., 1985](#)), which is why toxicological studies place greater emphasis on organ weight expressed relative to body weight. Whereas provision of L-Lys HCl and 0.30% LB elicited few changes in relative organ weights compared with the NC diet, we did observe what appeared to be LB dose-dependent increases in relative weights of the liver, spleen, pancreas, and ileum, all of which are important indicators of metabolic aberrations from a safety perspective. Specifically, the liver receives drained blood and metabolites from the digestive tract, spleen, and pancreas and therefore often shows the first signs of toxicity from compounds ([Ferreira et al., 2012](#)) and the spleen is majorly responsible for mounting adaptive immune responses and pathogen clearance ([Mebius and Kraal, 2005](#)). Enlargement of the spleen and liver and tissue lesions were observed in previous research that exposed avian species to toxic substances, and spleen enlargement was attributed to an influx of lymphocytes ([Takahashi and Kaya, 1993](#); [Karaman et al., 2005](#)). However, previous research found that supplementing essential AA can result in an enlargement of several organs including the liver and spleen likely due to enhanced metabolism ([Benevenga and Stelle, 1984](#); [Bartell and Batal, 2007](#)) or enhanced immune capacity ([Adjei et al., 1994](#)). Moreover, organ hypertrophy may be attributed to the increased deposition of iron (i.e., hemosiderin), especially in the liver and spleen, due to an increase in red blood cell turnover ([Benevenga and Stelle, 1984](#)). Beyond Lys, it is possible that other AA, nutrients, or compounds found in the LB product stimulated the growth of organ mass (e.g., intestinal mucosal mass; [Spector et al., 1977](#); [Inoue et al., 1993](#)), and pancreatic and biliary secretions are also known to mediate changes in small intestinal mass ([Gélinas and Morin, 1980](#)). As the relative weights of all measured organs of the 0.30% LB treatment—except the spleen—are similar to at least one of the control treatments, we believe these observations are likely due to enhanced metabolism and red blood cell turnover. In contrast, the relative weights for the majority of organs from birds receiving the 1.50% LB treatment were increased above both control treatments. Additionally, the 0.90% LB treatment elicited variable results for some organs, although we did not observe any visible tissue lesions in birds assigned to any treatment. As such, our observations of enlarged organs (and the aforementioned reduced growth effects) for the 2 higher supplementation levels of LB may indicate signs of toxicity. Therefore, this provides evidence that supplementation of the LB product at 0.30% in the diet restores growth performance to the level of the PC birds but at 0.90% and above is not advantageous to growth and well-being of broiler chickens.

To evaluate the effects of LB and the resultant increasing Lys levels more closely, blood parameters

were also assessed. In toxicity and safety studies, blood parameters are key factors in determining the health status of an animal and in identifying signs of internal damage (Weingand et al., 1992, 1996). There were no differences between treatments for the majority of the hematological parameters analyzed; however, reduced lymphocyte percentage was accompanied by a higher proportion of heterophils in the LB treatments, which may have implications on immunity. Lymphocytes and heterophils are cells of the immune system, and during acute inflammatory responses, heterophils are the predominant granulated leukocyte in avian species (Harmon, 1998). Broilers exposed to various stressors exhibit increased proportions of heterophils and decreased proportions of lymphocytes, which leads to an elevation of the H:L ratio (Martrenchar et al., 1997; Feddes et al., 2002; Maroufyan et al., 2010), though this response may be inconsistent (Al-Murrani et al., 2002). The release of corticosterone during times of stress has been linked to increased H:L ratios and increased weights of the liver, intestine, and adipose (Scanen, 2016).

Other than stress, such immune responses could also be attributed to the LB product containing killed cells of the bacteria *C. glutamicum*. Tong et al. (2012) found that killed bacteria cells influence the immune system in rodents and Ebisawa et al. (2017) found *C. glutamicum* to have an immune stimulating effect beneficial to mice challenged with disease. Although it may have an influence on the immune system, *C. glutamicum* is a feed ingredient with generally recognized as safe status. It is also known that supplementation of broilers with AA elicits an increase in heterophil percentage and decreased percentages of lymphocytes, therefore resulting in an increased H:L ratio (Jahanian, 2009; Bouyeh, 2012; Mahdavi et al., 2012). Interestingly, the changes in the H:L ratio observed in the current study did not appear to be dose-dependent, as birds fed the lowest level of LB were observed to have the highest H:L ratio. These findings suggest that the observed changes in heterophil and lymphocyte concentrations were likely in response to ingestion of the LB product, although not likely attributed directly to stress. Additional research is needed to investigate this outcome.

In conjunction with hematology, we also found differences in blood chemistry parameters. Glucose was highest in the 0.30% LB treatment; however, this may be due to error during sampling where all feeders were removed and all birds of a single treatment were collected and euthanized before progressing to the next treatment. Essentially, birds transitioned from a fed to a fasted state (Krestel-Rickert et al., 1986) as we progressed through treatments (while still blinded) in the following order: 0.30% LB, 1.50% LB, 0.90% LB, PC, NC. This may explain why the 0.30% LB treatment resulted in having the highest blood glucose levels, as these birds were still in a fully fed state at the time of sample collection. Conversely, Sekelova et al. (2017) describes a relationship where heterophils play a role in metabolism to increase glucose availability. Therefore, it is also possible

that the increased levels of heterophils in the LB-supplemented treatments are associated with elevated circulating concentrations of glucose in the same birds, specifically the 0.30% LB treatment. AST and CPK levels appeared to increase with increasing supplementation of LB, similar to our findings for liver and proventriculus weights, suggesting a possible interaction. Increased levels of AST in the serum are associated with liver and muscle disorder (Valentine et al., 1990), especially in terms of the AST to alanine aminotransferase ratio (Giannini et al., 1999), although alanine transferase was not measured in this study. Increased CPK levels have been associated with muscle tissue, heart, and brain damage in humans and animals (Bhavani et al., 2010). These elevated enzymes, indicative of muscle and liver damage, could explain why the birds fed 0.90 and 1.50% LB had decreased growth performance, although current research in birds is limited. Therefore, in conjunction with our conclusion based on organ weights, we determined that supplementation of the LB product at 0.90% or above has adverse effects on hematology and serum chemistry parameters in broilers.

In the current study, we found that supplementation of the LB product at 0.30% restored growth performance to the level of the PC birds supplemented with 0.23% L-Lys-HCl with minimal impact on metabolic indicators of safety. Conversely, we found supplementation of the LB product at 0.90% of the diet or higher to have a negative impact on growth performance and various clinical indicators in broilers, although more research is needed to investigate these relationships. Therefore, an inclusion rate of the LB product up to 0.30% may serve as a practical source of Lys in broiler diets.

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DISCLOSURES

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