Determination of the optimum digestible isoleucine to lysine ratios for male Yield Plus × Ross 708 broilers between 1.0 and 4.0 kg body weight utilizing growth performance and carcass characteristics[1]

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ABSTRACT Three experiments (**Exp**) were conducted to determine optimal digestible Ile to Lys ratios for male Yield Plus × Ross 708 broilers from approximately 1.0 to 4.0 kg BW. Broilers were fed dose-response diets with inclusions of blood cells that were formulated to contain a gradient of digestible Ile to Lys ratios (0.46 to 0.83). Treatments for Exp 1 to 3 were fed from 21 to 35, 28 to 42, and 35 to 49 d of age, respectively, to target market weights from 2.5 to 4.0 kg. Experiments utilized positive control (**PC**) diets that did not contain blood cells and were formulated to the same Ile ratios as Treatment 5. Birds and feed were weighed by pen on the first and last days of the experimental period to determine growth performance. Selected broilers were processed and deboned to determine carcass characteristics. For all Exp, quadratic effects ($P \leq 0.001$) were observed with BW gain, feed conversion ratio (**FCR**), breast meat weight, and breast meat yield (**BMY**) as digestible Ile to Lys ratios increased. Contrasts between PC and Treatment 5 for each Exp displayed no effect of blood cell inclusion with the exception of FCR in Exp 1 ($P = 0.001$) and BMY in Exp 3 ($P = 0.017$). Optimum digestible Ile to Lys ratios for Exp 1 were determined to range from 0.640 to 0.725 for growth from 1.0 to 2.5 kg BW ($P \leq 0.001$) and breast meat characteristics. In Exp 2, optimum ratios ranged from 0.664 to 0.682 for growth and breast meat characteristics from 1.6 to 3.1 kg BW ($P \leq 0.001$). For growth and breast meat characteristics of broilers in Exp 3, optimum ratios ranged from 0.625 to 0.730, from 2.6 to 3.9 kg BW ($P \leq 0.001$). Based on these findings, optimum digestible Ile to Lys ratios were determined to range from 0.63 to 0.73 for broilers from 1.0 to 4.0 kg BW.

Key words: isoleucine, ideal ratio, amino acid, broiler

INTRODUCTION

Dietary Ile is the 4th or 5th limiting amino acid (**AA**) in corn and soybean-meal based diets with inclusions of animal protein meals fed to broilers (Kidd et al., 2000; Corzo et al., 2009). Isoleucine is a member of a subgroup of AA known as branched-chain AA that also includes Val and Leu. The primary function of Ile is contributing to lean tissue development and is a substantial portion of edible tissue (Bae et al., 1999). Isoleucine is reported to play an important role in active immunity and the production of immune cells (Hale et al., 2004; Bender, 2012).

Digestible (**dig**) Ile requirements and optimum dig Ile to Lys ratios have been reported for broilers up to 1.0 kg BW (Farran and Thomas, 1990; Baker et al., 2002; Barbour and Latshaw, 1992; NRC, 1994; Baker, 1996, 1997). In addition, published research has reported total and dig Ile requirements and optimum dig Ile to Lys ratios for broilers from 1.0 to 3.5 kg BW (NRC, 1994; Baker, 1996; Kidd et al., 2004; Berres et al., 2010; Miranda et al., 2015). However, a disproportionate amount of the existing literature is available for broilers under 1.0 kg BW, and many of these published articles are over 15 yr old. It is now common practice to formulate poultry diets using ideal ratios of limiting AA with Lys as a reference AA (Baker, 2003); however, there is limited published research reporting optimum ratios for dig Ile in broilers greater than 1.0 kg.

Previous research has demonstrated that Ile responses for breast muscle accretion are greater than for growth

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Auburn University.

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(Kidd et al., 2004; Mejia et al., 2011; Dozier et al., 2012). However, published data are sparse reporting optimum dig Ile ratios for growth performance and breast meat yields of broilers grown greater than 2.5 kg. Therefore, determining optimum dig Ile to Lys ratios for broilers from 1.0 to 4.0 kg BW is paramount for accurately formulating broiler diets and optimizing meat yields of broilers across a range of market weights. Objectives of these experiments were to determine the optimum dig Ile to Lys ratios of broilers from 1.0 to 2.5, 1.5 to 3.0, and 2.5 to 4.0 kg BW utilizing responses of growth and carcass parameters.

MATERIALS AND METHODS

All procedures involving live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2018-3395, PRN 2019-3555, PRN 2020-3655)

Common Procedures

Three experiments (**Exp**) were conducted utilizing Yield Plus × Ross 708 male broiler chicks (Aviagen North America, Huntsville, AL) obtained from a commercial hatchery at 1 d of age. At the hatchery, all chicks received vaccinations for Marek's disease, Newcastle disease, and infectious bronchitis. Broiler chicks were placed into floor pens (Exp 1 = 72 pens, 30 birds/pen, 0.11 m^2/bird; Exp 2 = 64 pens, 25 birds/pen, 0.09 m^2/bird; Exp 3 = 72 pens, 30 birds/pen, 0.11 m^2/bird) of a solid-sided house. Each pen was equipped with a tube feeder, a nipple drinker line, and litter from a single previous flock. Experimental facilities consisted of a negative-pressure ventilation system equipped with vent boards, exhaust fans, evaporative cooling pads, and an electronic controller to maintain the temperature and ventilation needs of the birds. House temperature at chick placement was maintained at 33°C and was gradually reduced to 20°C at d 21 to maintain bird comfort. The photoperiod was set at 23L:1D for the first 7 d posthatch and 20L:4D was maintained for the duration of the Exp. Light intensity was set at 30, 10, and 5 lux from 1 to 7, 8 to 14, and 15 d of age through the duration of the Exp, respectively. Feed and water were provided ad libitum throughout the experimental periods. Broiler chicks were fed common starter and grower diets until the beginning of the experimental periods (Exp 1 = 1 to 20 d of age; Exp 2 = 1 to 14 and 15 to 27 d of age; Exp 3 = 1 to 18 and 19 to 34 d of age), formulated to meet or exceed the nutrient recommendations of the NRC (1994) (Table 1). The incidence of mortality was recorded daily throughout each Exp.

Dietary Treatments

Amino acid analysis of corn, soybean-meal, and spray-dried blood cells are presented in Table 2. Corn and

Table 1. Ingredient composition and calculated nutrient analysis for common starter and grower diets.

Ingredient, % "as-fed"	Starter	Grower
Corn	50.81	53.62
Soybean meal	40.88	38.29
Soybean oil	4.38	4.60
Defluorinated phosphate	1.89	1.63
Limestone	0.78	0.73
NaCl	0.46	0.47
DL-Methionine	0.30	0.29
AU vitamin premix[1]	0.10	0.10
AU trace mineral premix[2]	0.10	0.10
L-Threonine	0.10	0.05
L-Lysine-HCl	0.08	0.02
Choline-Cl[3]	0.08	0.08
IBCu (intelibond)[4]	—	0.02
Calculated analysis, % (unless otherwise noted)		
AME$_n$[5]	3,053	3,086
Crude protein	23.26	21.78
Digestible Lys	1.23	1.10
Digestible Met	0.64	0.58
Digestible Thr	0.84	0.75
Digestible TSAA	0.93	0.85
Calcium	1.01	0.90
Phosphorus-AV	0.48	0.43
Sodium	0.22	0.21

[1]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 9,370 IU; Vitamin D (cholecalciferol), 3,300 IU; Vitamin E (DL-alpha tocopheryl acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg; D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamin (thiamin mononitrate), 2.7 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.8 mg.

[2]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediaminedihydroxide), 1.4 mg; Se (sodium selenite), 0.3 mg.

[3]Choline chloride-70 (Balchem Corporation, New Hamptopn, NY).

[4]Tribasic Copper Chloride (Micronutrients, Indianapolis, IN).

[5]AMEn- nitrogen-corrected apparent metabolizable energy.

soybean meal were analyzed using near infrared reflective spectroscopy (Evonik Nutrition AMINO*NIR*®) to determine dig AA concentrations. Blood cells (American Protein Corporation, Arion, IA) were analyzed via HPLC (method 982.30 E (a,b,c); AOAC International, 2006) to determine the total AA content. Digestible AA content of the blood cells was determined by multiplying the total AA concentrations by digestibility coefficients adapted from the Brazilian Tables for Poultry and Swine (2017). Different batches of corn, soybean meal, and blood cells were used in each experiment (Table 2). Blood cells were used due to their low concentration of dig Ile (0.40 to 0.75%) relative to the other indispensable AA. This enabled a small inclusion of blood cells to create a dig Ile deficient test diet (Negative Control).

In Exp 1, 2, and 3, dig Ile to Lys ratios of titrated diets were formulated to range from 0.48 to 0.83, 0.46 to 0.82, and 0.48 to 0.83, respectively (Table 3). Experimental diets were created by the mixing of negative control (**NC**) (deficient in dig Ile) and summit (excess dig Ile) diets in varying proportions. Each experimental diet was formulated to contain 95% of the recommended dig Lys concentration to prevent birds from overconsuming Lys (Baker and Han, 1994; NRC, 1994). For Exp 1, the NC diet was formulated to contain 0.50% dig Ile and

Table 2. Analysis of amino acid and crude protein concentrations of primary ingredients used in the formulation of diets fed to Yield Plus × Ross 708 male broilers from 1.0 to 4.0 kg body mass.

	Corn		SBM[1]		Blood Cells[2]	
			Experiment 1[3]			
Nutrient, % "as fed"	Total[4]	Dig[4]	Total[4]	Dig[4]	Total[5]	Dig[6]
Crude protein	7.95	—	43.86	—	96.75	—
SAA	0.370	0.329	1.220	1.015	1.820	1.638
Lys	0.260	0.219	2.710	2.350	9.240	8.316
Thr	0.290	0.244	1.750	1.440	4.400	3.960
Val	0.400	0.359	2.160	1.827	9.100	8.190
Ile	0.290	0.270	2.090	1.814	0.410	0.369
Trp	0.080	0.067	0.600	0.521	1.680	1.512
Arg	0.380	0.337	3.090	2.818	3.600	3.240
His	0.230	0.214	1.120	0.993	6.410	5.769
Phe	0.400	0.367	2.210	1.960	7.540	6.786
Leu	0.970	0.895	3.370	2.956	13.000	11.700
			Experiment 2			
Crude protein	8.50	—	47.75	—	93.67	—
SAA	0.335	0.281	1.250	1.062	1.736	1.562
Lys	0.261	0.201	2.862	2.518	8.518	7.667
Thr	0.273	0.216	1.798	1.528	4.196	3.776
Val	0.364	0.309	2.118	1.864	7.783	7.005
Ile	0.261	0.225	2.077	1.807	0.833	0.750
Trp	0.047	0.039	0.672	0.584	1.587	1.428
Arg	0.375	0.326	3.358	2.955	3.364	3.028
His	0.205	0.174	1.178	1.013	5.776	5.198
Phe	0.341	0.303	2.438	2.170	6.343	5.709
Leu	0.898	0.826	3.533	3.145	11.820	10.638
			Experiment 3			
Crude protein	7.11	—	46.56	—	90.00	—
SAA	0.320	0.278	1.260	1.064	1.900	1.710
Lys	0.240	0.197	2.820	2.482	8.520	7.668
Thr	0.255	0.209	1.780	1.495	4.200	3.780
Val	0.340	0.299	2.200	1.936	5.000	4.500
Ile	0.245	0.216	2.110	1.836	0.690	0.621
Trp	0.055	0.042	0.630	0.554	1.450	1.305
Arg	0.360	0.317	3.350	3.015	3.400	3.060
His	0.210	0.189	1.200	1.056	5.580	5.265
Phe	0.330	0.297	2.380	2.118	6.860	6.174
Leu	0.830	0.772	3.500	3.080	12.110	10.899

[1]Soybean meal.

[2]American Protein Corporation, Arion, IA.

[3]Broilers in were fed experimental diets from 21 to 35, 28 to 42, and 35 to 49 d of age for Experiments 1, 2, and 3, respectively.

[4]Digestible values determined using Evonik Nutrition AMINO*NIR*® near infrared reflective spectroscopy.

[5]Values obtained from HPLC analysis of ingredients (method 982.30 E (a,b,c); AOAC International, 2006).

[6]Values obtained by multiplying total amino acid concentrations by digestibility coefficients adapted from the Brazilian Tables (2017) for blood cells.

1.05% dig Lys, and the summit was formulated to contain 0.87 and 1.05% dig Ile and Lys, respectively. Treatments 1 to 8 contained 100.0, 85.7, 71.4, 57.1, 42.9, 28.6, 14.3, 0.0% NC diet, respectively, and the remaining proportion of each treatment (**Trt**) was comprised of the summit diet. In Exp 2, the NC diet was formulated to contain 0.44% dig Ile and 0.95% dig Lys, and the summit diet was formulated to contain 0.78% dig Ile and 0.95% dig Lys. Diets were consisted of 100.0, 83.3, 66.7, 50.0, 33.3, 16.7, 0.0% NC diet for Trt 1 to 7, respectively, and the summit diet comprised the remainder. In Exp 3, the NC diet was formulated to contain 0.42 and 0.87% dig Ile and Lys, respectively, and the summit was formulated to contain 0.72 and 0.87% dig Ile and Lys, respectively. Treatments 1 to 8 contained 100.0, 85.7, 71.4, 57.1, 42.9, 28.6, 14.3, 0.0% NC diet, respectively, and the remainder was made up of summit diet. All Exp had a positive control (**PC**) (Exp 1 = Trt 9; Exp 2 = Trt 8; Exp 3 = Trt 9) that was formulated without blood cells to have the same dig Ile to Lys ratio as Trt 5. For Exp 1,

2, and 3, PC diets were formulated to contain dig Ile to Lys ratios of 0.68, 0.70, and 0.68, respectively.

Measurements

Birds and feed were weighed by pen at the beginning and end of each experimental period (Exp 1 = 21 and 35 d of age; Exp 2 = 28 and 42 d of age; Exp 3 = 35 and 49 d of age) in order to determine BW gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**). At the end of each experimental period (Exp 1 = d 36; Exp 2 = d 43; Exp 3 = d 50), birds were randomly selected to be processed to assess carcass characteristics (Exp 1 = 9 birds/pen; Exp 2 = 14 birds/pen; Exp 3 = 14 birds/pen). Birds were processed in a pilot processing facility at the Auburn University Poultry Research Unit following a 12-h feed withdrawal period. Broilers were electronically stunned, exsanguinated, scalded, picked mechanically, eviscerated mechanically, and placed on ice. Carcasses were chilled in ice water for a period of 3 h

Table 3. Ingredient and nutrient composition of dietary treatments fed to Yield Plus × Ross 708 male broilers from 1.0 to 4.0 kg body mass[1].

Ingredient, % "as-fed"	Experiment 1[2]			Experiment 2			Experiment 3		
	NC[3]	Summit	PC[4]	NC	Summit	PC[4]	NC	Summit	PC[4]
Corn	76.25	76.07	62.46	79.10	78.95	64.28	81.10	80.93	69.80
Soybean meal	15.52	15.52	29.99	12.92	12.52	28.81	11.15	11.15	24.03
Soybean oil	0.45	0.25	3.12	0.45	0.25	3.57	0.91	0.77	3.32
Limestone	0.67	0.67	0.68	0.66	0.66	0.60	0.81	0.81	0.79
Defluorinated phosphate	1.00	1.00	0.98	1.49	1.49	1.29	0.84	0.85	0.76
Sodium bicarbonate	0.27	0.27	0.29	0.34	0.34	0.32	0.37	0.37	0.37
Salt, NaCl	0.18	0.18	0.21	0.17	0.17	0.23	0.18	0.18	0.25
DL-Methionine	0.43	0.43	0.35	0.39	0.39	0.30	0.33	0.33	0.23
L-Lysine, HCl	0.40	0.40	0.27	0.40	0.40	0.12	0.33	0.33	0.17
AU Trace mineral premix[5]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Threonine	0.23	0.23	0.15	0.23	0.23	0.09	0.18	0.18	0.08
AU vitamin premix[6]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Phytase[7]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L-Val	0.11	0.11	0.09	0.16	0.16	0.05	0.16	0.16	0.005
L-Ile	0.01	0.38	—	0.01	0.36	—	0.02	0.33	—
L-Trp	0.05	0.05	0.01	0.08	0.08	0.01	0.04	0.04	—
L-Arg	0.60	0.60	0.31	0.42	0.42	0.06	0.30	0.30	—
Gly	0.68	0.69	0.41	0.50	0.50	0.09	0.32	0.32	—
Blood cells[8]	2.50	2.50	—	2.00	2.00	—	2.25	2.25	—
Titanium dioxide	0.50	0.50	—	0.50	0.50	—	0.50	0.50	—
Calculated analysis, % (unless otherwise noted)									
AME, kcal/kg	3,120	3,120	3,120	3,155	3,152	3,152	3,196	3,196	3,196
Crude protein	18.09	18.32	19.88	17.17	17.39	19.84	15.53	15.71	17.19
Digestible Lys[9]	1.05	1.05	1.05	0.95	0.95	0.95	0.87	0.87	0.87
Digestible SAA[10]	0.87	0.87	0.86	0.78	0.78	0.78	0.71	0.71	0.68
Digestible Thr	0.74	0.74	0.74	0.67	0.67	0.67	0.60	0.60	0.58
Digestible Val	0.87	0.87	0.86	0.78	0.78	0.78	0.71	0.71	0.68
Digestible Ile[11]	0.50	0.87	0.71	0.44	0.78	0.67	0.42	0.72	0.59
Digestible Leu	1.44	1.44	1.45	1.28	1.28	1.44	1.22	1.22	1.28
Ca[12]	0.76	0.76	0.76	0.90	0.90	0.86	0.76	0.76	0.76
Nonphytate P	0.40	0.40	0.40	0.43	0.43	0.43	0.38	0.38	0.38
Analyzable P[13]	0.48	0.48	0.51	0.57	0.57	0.61	0.45	0.45	0.49
Na	0.21	0.21	0.21	0.23	0.23	0.23	0.23	0.23	0.23

[1]Experimental diets 1 to 8 for experiments 1 and 3 contained 100.0, 85.7, 71.4, 57.1, 42.9, 28.6, 14.3, 0.0% NC diet, respectively and diets 1 to 7 in experiment 2 contained 100.0, 83.3, 66.7, 50.0, 33.3, 16.7, 0.0% NC diet, respectively, and remaining space in all diets was comprised of summit diet to create diets of intermediate digestible Ile to Lys ratios.

[2]Broilers were fed experimental diets from 21 to 35, 28 to 42, and 35 to 49 d of age for Experiments 1, 2, and 3, respectively, in 8 replications/treatment.

[3]Negative control.

[4]Positive control diets formulated to have a digestible Ile to Lys ratio of 0.68, 0.70, and 0.68 for Experiments 1, 2, and 3, respectively.

[5]Trace mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg;Cu (tribasic copper chloride), 8 mg; I (ethylenediamine dihydriodide), 1.4 mg; and Se (sodium selenite), 0.3 mg.

[6]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D3(cholecalciferol), 6,614 IU; Vitamin E (DL-alphatocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12(cyanocobalamin), 0.03 mg; folacin (folic acid), 2.6mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22 mg; niacin (niacinamide), 88 mg; thiamin (thiamin mononitrate),5.5 mg; biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[7]Quantum Blue Phytase, AB Vista, Marlborough, UK.

[8]American Protein Corporation, Arion, IA.

[9]Analyzed digestible Lys concentrations were 0.94 and 0.92, 0.94 and 0.92, and 0.92 and 0.84% for negative control and summit diets of Experiments 1 to 3, respectively.

[10]Sulfer Amino Acids.

[11]Analyzed digestible Ile concentrations were 0.48 and 0.68, 0.39 and 0.77, and 0.42 and 0.62% for negative control and summit diets of Experiments 1 to 3, respectively.

[12]Analyzed Ca values were determined as 0.72, 0.72, and 0.75% For Exp 1; 0.91, 0.89, and 0.86% for Exp 2; and 0.75, 0.74, and 0.76% for Exp 3.

[13]Analyzable P values were determined as 0.46, 0.46, and 0.51% For Exp 1; 0.57, 0.56, and 0.60% for Exp 2; and 0.43, 0.43, and 0.48% for Exp 3.

and then drained of excess water for approximately 3 min. The abdominal fat pad was removed and weighed separately from the chilled carcass to determine the fat percentage. Carcasses were deboned the following day to obtain breast fillets (*pectoralis major*), tenders (*pectoralis minor*), wings, drums, and boneless-skinless thigh meat by experienced personnel utilizing stationary cones (Exp 1). For Exp 2 and 3, breast fillets and tenders were weighed. Tender and breast fillet weights were combined for the analysis of total breast meat weight. Meat yield percentages were based on live weight at 35, 42, and 49 d of age for Exp 1, 2, and 3, respectively.

Apparent Ileal Amino Acid Digestibility Assays

For all Exp, apparent ileal AA digestibility was determined for the NC and summit diets. At 38, 45, and 52 d of age for Exp 1, 2 and 3, respectively, ileal digesta was collected from 6 birds per pen from the NC and summit Trt (Exp 1 = treatments 1 and 8; Exp 2 = treatments 1 and 7; Exp 3 = treatments 1 and 8). Birds were euthanized via CO_2 asphyxiation and digesta was collected by gently flushing a section of the terminal ileum (terminal 1/3 between the Meckel's diverticulum and 2 cm

Table 4. Amino acid digestibility for male Yield Plus × Ross 708 broilers fed negative control and summit diets from 1.0 to 4.0 kg body mass[1].

| | Experiment 1[2] | | | | Experiment 2 | | | | Experiment 3 | | | |
| | NC[3] | | Summit[4] | | NC | | Summit | | NC | | Summit | |
Amino acid	DC[5] %	dig AA[6] %	DC %	dig AA %	DC %	dig AA %	DC %	dig AA %	DC %	dig AA %	DC %	dig AA %
Lys	89.85	1.01	89.73	1.00	90.31	0.94	90.07	0.92	91.29	0.92	90.73	0.88
SAA[7]	88.69	0.70	88.51	0.70	93.81	0.70	93.57	0.72	91.27	0.71	90.48	0.68
Thr	82.40	0.66	82.55	0.66	83.62	0.65	82.07	0.60	84.73	0.60	83.55	0.58
Val	85.82	0.77	85.36	0.78	86.47	0.73	86.81	0.79	87.67	0.78	86.38	0.78
Ile	80.01	0.45	86.99	0.72	80.43	0.39	88.37	0.77	82.87	0.41	88.26	0.65
Trp	83.21	0.18	82.21	0.18	86.39	0.21	85.67	0.20	86.23	0.15	87.77	0.15
Arg	93.31	1.30	92.78	1.29	91.57	1.05	91.70	1.09	93.45	0.99	92.54	0.96
Leu	86.79	1.32	87.31	1.33	87.50	1.22	87.43	1.32	87.83	1.22	86.02	1.21
Phe	88.13	0.78	87.31	0.75	87.11	0.63	86.83	0.65	88.64	0.70	86.98	0.67
His	88.19	0.43	87.27	0.42	89.52	0.38	89.34	0.39	88.95	0.40	87.32	0.39

[1]Experimental diets 1 to 8 for experiments 1 and 3 contained 100.0, 85.7, 71.4, 57.1, 42.9, 28.6, 14.3, 0.0% NC diet, respectively and diets 1 to 7 in experiment 2 contained 100.0, 83.3, 66.7, 50.0, 33.3, 16.7, 0.0% NC diet, respectively, and remaining space in all diets was comprised of summit diet to create diets of intermediate digestible Ile to Lys ratios.

[2]Broilers in were fed experimental diets from 21 to 35, 28 to 42, and 35 to 49 d of age for Experiment 1, Experiment 2, and Experiment 3, respectively in 8 replication/treatment.

[3]Negative control diets were formulated to contain 1.05% Lys and 0.50% Ile; 0.95% Lys and 0.44% Ile; and 0.87% Lys and 0.42% Ile on a digestible basis for Experiments 1 to 3, respectively.

[4]Summit diets were formulated to contain 1.05% Lys and 0.87% Ile; 0.95% Lys and 0.78% Ile; and 0.87% Lys and 0.72% Ile on a digestible basis for Experiments 1 to 3, respectively.

[5]Digestibility coefficient, obtained by titanium dioxide assay according to method of Short et al. (1996).

[6]Concentration of digestible amino acid included in the diet. Obtained by multiplying total amino acid concentration from HPLC analysis by the digestibility coefficient.

[7]Sulfur Amino Acids.

proximal to the ileo-cecal junction) with deionized water. Diets and digesta were lyophilized in a Virtis Genesis Pilot Lyophilizer (SP Industries, Warminster, PA), and then ground in an electric coffee grinder (Hamilton Beach, Glen Allen, VA). The dried digesta was analyzed in duplicate and dried diets were analyzed in quadruplicate for TiO_2 concentration using the method described by Short et al. (1996). Absorbance was measured on a spectrophotometer (SPECTRAmax Plus 384, Molecular Devices, Sunnyvale, CA), using 1.0 mL of solution in a cuvette reader. A standard curve was used to create a regression equation (R^2 = 0.991, 0.985, and 0.990 for Exp 1, 2, and 3, respectively) as a reference to calculate TiO_2 concentrations in diets and digesta. Digesta and diet samples were also analyzed in duplicate for AA profile using HPLC (method 982.30 E (a,b,c); AOAC International, 2006). These values were used to calculate apparent ileal AA digestibility using the following formula:

$$AIAAD\ \% = \left[1 - \left(\frac{Ti_i}{Ti_o} \right) \times \left(\frac{AA_o}{AA_i} \right) \right] \times 100$$

where Ti_i represents the TiO_2 concentration in the input (diet), Ti_o represents the TiO_2 concentration in the output (digesta), AA_o represents the concentration of the AA in the output, and AA_i represents the concentration of the AA in the input (Dilger et al., 2004).

Statistical Analysis

All 3 Exp were conducted as a randomized complete block design with pen location as the blocking factor and 8 replications/trt. Pen was considered the experimental unit. Regression analysis and contrasts were performed with the PROC REG and PROC MIXED procedures of SAS 9.4 (2017). The dose response dietary Trt (Exp 1 = 1 to 8; Exp 2 = 1 to 7; Exp 3 = 1 to 8) were delineated for optimum dig Ile to Lys ratios using linear and quadratic regression. For all Exp, a contrast was performed between the PC and Trt 5 in order to determine the effects of blood cell inclusion, as these diets were formulated to contain the same dig Ile to Lys ratio. For these analyses, mortality was arcsine of the square root transformed. The determination of the optimum ratios for all Exp was performed via linear and quadratic broken-line regression using Programa Prático de Modelagem (Garcia-neto and Perri, 2015) on the dose response diets. For all statistical processes, significance was considered at P-value $\leq$ 0.05.

RESULTS AND DISCUSSION

For Exp 1, analyzed dig Lys and Ile values of test diets were lower than the formulated concentrations (Table 4). For Exp 2, analyzed dig Lys values for the test diets were in agreement with the calculated values as was the dig Ile concentration of the summit; however, analyzed values for Ile in the NC were lower than formulated (Table 4). Analyzed dig Lys concentrations for Exp 3 were in agreement with calculated values as was the dig Ile concentration in the NC; however, the analyzed Ile concentration of the summit was lower than formulated. These discrepancies in analyzed dig Ile values may be attributed to the lower digestibility coefficients of the NC diets as Table 4 illustrates that the digestibility coefficients of Ile are considerably lower for

Table 5. Growth performance of male Yield Plus × Ross 708 broilers fed varying digestible Ile to Lys ratios from 1.0 to 2.5 kg BW (21 to 35 d of age)[1].

Dietary treatments[2]	BW (kg)	BWG[3] (kg)	FI[4] (mg/d)	Dig Ile Intake[5] (kg/kg)	FCR[6] (%)	Mortality
1) dIle:Lys ratio 0.44	2.063	1.082	2.051	613	1.897	0.42
2) dIle:Lys ratio 0.49	2.199	1.198	2.123	679	1.772	0.86
3) dIle:Lys ratio 0.54	2.389	1.355	2.254	766	1.663	0.86
4) dIle:Lys ratio 0.59	2.451	1.437	2.302	852	1.602	0.00
5) dIle:Lys ratio 0.64	2.498	1.484	2.334	935	1.573	0.86
6) dIle:Lys ratio 0.70	2.467	1.465	2.283	964	1.558	0.45
7) dIle:Lys ratio 0.75	2.471	1.459	2.262	1,012	1.551	0.83
8) dIle:Lys ratio 0.81	2.490	1.493	2.292	1,108	1.536	0.00
9) Positive control (PC)[7]	2.473	1.446	2.190	963	1.515	1.82
SEM[8]	0.027	0.015	0.022	8	0.009	0.026
Regression analysis	—	—	—	Probabilities —	—	—
Linear	0.001	0.001	0.001	0.001	0.001	0.59
Quadratic	0.001	0.001	0.001	0.001	0.001	0.74
Contrast						
PC vs. Trt 5	0.33	0.08	0.001	0.022	0.001	0.18
	—	—	— Coefficient of Determination —	—	—	—
R^2 linear	0.673	0.711	0.395	0.968	0.782	0.005
R^2 quadratic	0.848	0.905	0.648	0.972	0.944	0.010

[1]Values represent least-square means for 8 replicate pens with 30 chicks per pen at 35 d of age. Experiment 1.
[2]Treatments 1 to 8 are represented by calculated digestible Ile to Lys ratios.
[3]Body weight gain.
[4]Feed intake.
[5]Determined using digestible Ile concentration of each treatment.
[6]Feed conversion ratio corrected for mortality.
[7]Calculated digestible Ile to Lys ratio = 0.68.
[8]Pooled standard error.

the NC relative to the summit for all 3 Exp. These diets were formulated with a single assumed digestibility coefficient of Ile in blood cells (Rostagno, 2017); however, the progressive additions of L-Ile increased the digestibility of Ile in the diets as a smaller percentage of Ile originated from intact proteins but was rather derived from feed-grade Ile. These variations in dig Ile may also be due in part to the variation observed in soybean meal below the analyzed AA profile. Titanium recoveries for all experiments diets were between 97 and 101%.

Experiment 1

Positive linear and quadratic effects ($P < 0.001$) were observed for BW and BWG as dig Ile to Lys ratios increased in the diet for broilers approaching 2.5 kg BW (Table 5). Similarly, FI increased linearly and quadratically ($P < 0.001$) as dietary Ile ratios increased. Broilers fed increasing dig Ile to Lys ratios displayed linear and quadratic decreases in FCR ($P < 0.001$) when reaching an approximate end weight of 2.5 kg. Positive linear and quadratic effects were also observed ($P < 0.001$) for dig Ile intake. There was no effect ($P = 0.74$) of dietary dig Ile to Lys ratios on mortality. The contrast elucidated greater responses for birds provided Trt 5 relative to the PC for FI ($P < 0.001$) and FCR ($P < 0.001$), while daily dig Ile intake was greater ($P = 0.022$) for broilers fed the PC. No differences ($P > 0.05$) were observed between the PC and Trt 5 for BW, BWG, or mortality.

Positive linear and quadratic effects ($P < 0.001$) were observed for carcass weight, carcass yield, breast meat weight (**BMW**), and breast meat yield (**BMY**) as dig Ile to Lys ratios increased in the diet (Table 6). Similarly, drum weight, thigh meat weight, drum yield, and

thigh meat yield had linear and quadratic responses ($P < 0.001$) to increasing dietary dig Ile to Lys ratios. For broilers being fed progressive additions of dig Ile, increasing linear and quadratic responses of wing weight were observed ($P < 0.001$); however, there was no effect on wing yield ($P = 0.77$). Abdominal fat percentage displayed negative linear and quadratic effects ($P < 0.001$) as dig Ile to Lys ratios increased in the diet; however, there was no effect of dietary Ile to Lys ratio on abdominal fat weight ($P = 0.64$). The contrast illuminated differences between the PC and Trt 5 for wing yield (PC > Trt 5) and abdominal fat weight and yield (PC < Trt 5) ($P = 0.020$). There were no differences observed for any of the other carcass parameters ($P > 0.05$).

The optimum dig Ile to Lys ratio for BWG was determined to be 0.67 for broilers from 1.0 to 2.5 kg BW ($P < 0.001$) utilizing quadratic broken-line regression (Table 7). For FCR, the optimum dig Ile to Lys ratio was determined as 0.69 ($P < 0.001$) utilizing quadratic broken-line regression analysis. The optimum dig Ile to Lys ratio for BMY of broilers was estimated at 0.67 ($P < 0.001$) with linear broken-line analysis. A greater optimum dig Ile to Lys ratio for BMW was determined as 0.71 ($P < 0.001$) for broilers from 21 to 35 d of age with quadratic analysis.

Kidd et al. (2004) reported the total Ile requirement to range from 0.71 to 0.76% for growth performance and carcass characteristics from 18 to 30 d of age with a total Lys concentration of 1.09%. However, the reported daily total Ile intakes to reach optimum performance, 750 to 850 mg/d, were much lower than responses obtained in the current study (950 to 1,000 mg/d for growth performance and BMW; 1,050 mg/d for BMY on a dig basis). This deviation indicates that more dietary Ile may be

Table 6. Carcass characteristics of male Yield Plus × Ross 708 broilers fed diets with increasing digestible Ile to Lys ratios from 1.0 to 2.5 kg BW (21 to 35 d of age)[1].

	BW kg	Carcass Weight kg	Carcass Yield %	Breast meat Weight kg	Breast meat Yield %	Wing Weight kg	Wing Yield %	Drum Weight kg	Drum Yield %	Thigh meat Weight Kg	Thigh meat Yield %	Abdominal Fat Weight kg	Abdominal Fat Yield %
Response parameter													
Dietary treatments[2]													
1) dIle:Lys ratio 0.44	2.063	1.500	72.69	0.466	22.57	0.161	7.79	0.198	9.60	0.206	9.98	0.021	1.36
2) dIle:Lys ratio 0.49	2.199	1.598	72.67	0.514	23.36	0.168	7.66	0.206	9.40	0.216	9.81	0.021	1.33
3) dIle:Lys ratio 0.54	2.389	1.754	73.45	0.581	24.32	0.180	7.54	0.215	9.01	0.232	9.71	0.025	1.44
4) dIle:Lys ratio 0.59	2.451	1.799	73.38	0.610	24.86	0.189	7.71	0.223	9.08	0.241	9.81	0.022	1.21
5) dIle:Lys ratio 0.64	2.498	1.855	74.25	0.646	25.85	0.191	7.66	0.223	8.92	0.241	9.64	0.021	1.13
6) dIle:Lys ratio 0.70	2.467	1.820	73.77	0.644	26.11	0.189	7.66	0.221	8.94	0.237	9.61	0.020	1.08
7) dIle:Lys ratio 0.75	2.471	1.844	74.62	0.650	26.30	0.200	7.58	0.220	8.90	0.238	9.64	0.021	1.12
8) dIle:Lys ratio 0.81	2.490	1.859	74.67	0.656	26.39	0.193	7.74	0.224	8.97	0.240	9.65	0.022	1.18
9) Positive control (PC)[3]	2.473	1.836	74.21	0.638	25.77	0.194	7.83	0.223	9.00	0.232	9.36	0.015	0.84
SEM[4]	0.027	0.023	0.34	0.010	0.26	0.004	0.05	0.003	0.07	0.004	0.13	0.001	0.05
Regression analysis						Probabilities							
Linear	0.001	0.001	0.001	0.001	0.001	0.001	0.77	0.001	0.001	0.001	0.001	0.47	0.001
Quadratic	0.001	0.001	0.001	0.001	0.001	0.001	0.58	0.001	0.001	0.001	0.001	0.64	0.001
Contrast													
PC vs. Trt 5	0.49	0.55	0.95	0.83	0.81	0.70	0.020	0.89	0.43	0.13	0.12	0.001	0.001
						Coefficient of Determination							
R^2 linear	0.586	0.623	0.349	0.743	0.684	0.423	0.002	0.418	0.538	0.367	0.236	0.007	0.279
R^2 quadratic	0.796	0.785	0.350	0.870	0.723	0.495	0.018	0.570	0.644	0.518	0.252	0.014	0.305

[1]Values are least-square means of 8 replicate pens, with 9 birds/pen being selected and processed at d 35. Experiment 1.
[2]Treatments 1 to 8 are represented by calculated digestible Ile to Lys ratios.
[3]Calculated digestible Ile to Lys ratio = 0.68.
[4]Pooled standard error.

Table 7. Optimal digestible Ile to Lys ratios for male Yield Plus × Ross 708 broilers based on growth performance and carcass characteristics from 1.0 to 4.0 kg body mass.

Response	Estimated ratio[1]	95% CI[2]	SEM[3]	R^2 (%)	P-value
			Experiment 1[4]		
Linear					
BWG[5], kg	0.600	0.586 to 0.612	0.006	92.27	<0.001
FCR, kg:kg	0.605	0.594 to 0.617	0.007	93.54	<0.001
TBMW[6], kg	0.622	0.601 to 0.644	0.011	87.17	<0.001
TBMY[7], %	0.668	0.637 to 0.699	0.016	81.35	<0.001
Quadratic					
BWG, kg	0.668	0.639 to 0.697	0.015	92.07	<0.001
FCR, kg:kg	0.685	0.662 to 0.708	0.012	95.30	<0.001
TBMW, kg	0.713	0.668 to 0.757	0.023	87.26	<0.001
TBMY, %	0.806	0.722 to 0.890	0.043	81.14	<0.001
			Experiment 2		
Linear					
BWG, kg	0.665	0.609 to 0.722	0.029	96.03	<0.001
FCR, kg:kg	0.671	0.617 to 0.725	0.028	96.66	<0.001
TBMW, kg	0.664	0.627 to 0.701	0.019	91.90	<0.001
TBMY, %	0.682	0.590 to 0.775	0.047	92.28	<0.001
Quadratic					
BWG, kg	0.802	0.621 to 0.984	0.092	91.46	<0.001
FCR, kg:kg	0.808	0.644 to 0.979	0.083	92.58	<0.001
TBMW, kg	0.783	0.649 to 0.917	0.068	95.86	<0.001
TBMY, %	0.755	0.550 to 0.959	0.104	88.35	0.002
			Experiment 3		
Linear[8]					
BWG, kg	0.625	0.568 to 0.683	0.029	98.63	< 0.001
FCR, kg:kg	0.692	0.623 to 0.762	0.035	91.92	< 0.001
TBMW, kg	0.694	0.623 to 0.765	0.036	91.90	< 0.001
TBMY, %	0.730	0.652 to 0.807	0.039	92.81	< 0.001

[1]Values obtained using linear and quadratic broken-line modelling.
[2]95% confidence intervals for the optimal digestible Ile to Lys ratios.
[3]Standard error of the estimate.
[4]Broilers in were fed experimental diets from 21 to 35, 28 to 42, and 35 to 49 d of age for Experiments 1, 2, and 3, respectively.
[6]Total breast meat weight.
[7]Total breast meat yield.
[8]Quadratic broken-line regression did not fit the data for Experiment 3 and yielded insignificant results.

Table 8. Growth performance of male Yield Plus × Ross 708 broilers fed varying digestible Ile to Lys ratios from 1.6 to 3.1 kg BW (28 to 42 d of age)[1].

Dietary treatments[2]	BW (kg)	BWG[3] (kg)	FI[4] (kg)	Dig Ile Intake[5] (mg/d)	FCR[6] (kg/kg)	Mortality (%)
1) dIle:Lys ratio 0.42	2.732	1.178	2.279	903	1.940	4.0
2) dIle:Lys ratio 0.49	2.735	1.195	2.286	1,232	1.920	4.0
3) dIle:Lys ratio 0.56	2.939	1.377	2.436	1,437	1.771	3.5
4) dIle:Lys ratio 0.63	3.063	1.486	2.536	1,516	1.707	1.0
5) dIle:Lys ratio 0.69	3.117	1.521	2.533	1,718	1.665	1.0
6) dIle:Lys ratio 0.76	3.144	1.557	2.557	1,841	1.643	3.0
7) dIle:Lys ratio 0.83	3.097	1.514	2.518	1,948	1.665	3.5
8) Positive control (PC)[7]	3.119	1.541	2.472	1,862	1.605	1.5
SEM[8]	0.036	0.028	0.034	20.56	0.022	0.017
Regression analysis				Probabilities		
Linear	0.001	0.001	0.001	0.001	0.001	0.60
Quadratic	0.001	0.001	0.001	0.001	0.001	0.39
Contrast						
PC vs. Trt 5	0.98	0.57	0.18	0.001	0.051	0.84
				Coefficient of determination		
R^2 linear	0.598	0.650	0.427	0.938	0.657	0.007
R^2 quadratic	0.668	0.733	0.510	0.962	0.732	0.002

[1]Values represent least-square means for 8 replicate pens with 25 chicks per pen at 42 d of age. Experiment 2.
[2]Treatments 1 to 7 are represented by analyzed digestible Ile to Lys ratios.
[3]Body weight gain.
[4]Feed intake.
[5]Determined using digestible Ile concentration of each treatment.
[6]Feed conversion ratio corrected for mortality.
[7]Calculated digestible Ile to Lys ratio = 0.70.
[8]Pooled standard error.

required to obtain optimum growth responses in modern broiler strains. Elevated concentrations of dig Ile increased meat yields for all carcass parts except for wings. The inclusion of blood cells to Trt 5 may have caused an elevated FI response relative to the PC. There was no effect on the growth rate of the broilers, thus the lower FI caused broilers provided the PC diet to have a lower FCR than those fed Trt 5. Birds fed Trt 5 had lower daily dig Ile intakes when compared with the broilers consuming the PC even with the greater FI of birds caused by Trt 5 containing a lower concentration of dig Ile than it was formulated to contain. There were also differences observed for abdominal fat weight and yield, with broilers provided Trt 5 having the greater responses for these parameters relative to the PC. This may be due to broilers fed Trt 5 having a greater FI and a larger nutrient intake than birds provided the PC.

Optimum dig Ile to Lys ratios were 0.67 and 0.69 for BWG and BMW, respectively, above a previously reported optimum of 0.65 for broilers from 14 to 35 d of age (Berres et al., 2010). The optimum dig Ile to Lys ratio obtained for BMW of 0.71 is also greater than previously reported optimum dig Ile ratios for broilers in this age range (Baker et al., 2002; Baker, 1997). In other research, broilers of larger weight classes displayed estimates of optimum ratios that were greater for breast meat yield than for BWG (Mejia et al., 2011; Miranda et al., 2015). Mejia et al. (2011) reported an optimum dig Ile to Lys ratio of 0.72 for BMY, however, these data evaluated broilers from 1.5 to 3.1 kg BW. These data are in agreement with the value obtained in the present Exp for BMW, but the optimum Ile ratio for BMY was found to be lower at 0.67. This lower value for breast yield may be due to the relative size of the broilers, not

showing a difference in the dig Ile need between breast yield and growth.

Quadratic broken-line regression was used to determine optimum Ile ratios for BWG, FCR, and BMW. Both quadratic and linear broken-line models produced good fits to the data, with very similar standard errors and confidence intervals. The quadratic models were chosen for these parameters as when both linear and quadratic models are fit to curvilinear data the linear model can underestimate the optimum response (Robbins et al., 2006). The linear model was chosen to estimate the optimum ratio for BMY, as it produced a much lower standard error (0.016 vs. 0.043) and therefore a smaller 95% confidence interval. This may have occurred because there were not adequate additional data points above the break point of the fit line causing an over estimation of the optimum ratio with the quadratic model. Based on these data, an optimal dig Ile to Lys ratio of 0.67 to 0.69 is likely appropriate for broilers from 1.0 to 2.5 kg BW.

Experiment 2

Positive linear and quadratic responses were observed ($P < 0.001$) for BW and BWG as dig Ile to Lys ratios increased in the diet for broilers with an approximate ending weight of 3.1 kg (Table 8). Similarly, FI and daily dig Ile intake displayed increasing linear and quadratic effects ($P < 0.001$) as dig Ile to Lys ratios were elevated from Trt 1 to 7. The FCR of broilers that consumed increasing dig Ile to Lys ratios were observed to decrease linearly and quadratically ($P < 0.001$). No effect ($P = 0.60$) of dietary dig Ile to Lys ratios was noted for

Table 9. Carcass characteristics of male Yield Plus × Ross 708 broilers fed diets with increasing digestible Ile to Lys ratios from 1.6 to 3.1 kg BW (28 to 42 d of age)[1].

Response parameter	Live Weight, kg	Carcass		Breast meat		Abdominal Fat	
		Weight, kg	Yield, %	Weight, kg	Yield, %	Weight, kg	Percentage, %
Dietary treatments[2]							
1) dIle:Lys ratio 0.42	2.720	2.028	74.57	0.685	25.19	0.026	0.94
2) dIle:Lys ratio 0.49	2.790	2.081	74.60	0.708	25.41	0.026	0.92
3) dIle:Lys ratio 0.56	2.982	2.240	75.22	0.786	26.38	0.027	0.92
4) dIle:Lys ratio 0.63	3.112	2.348	75.50	0.831	26.70	0.032	1.04
5) dIle:Lys ratio 0.69	3.137	2.385	75.91	0.861	27.56	0.030	0.95
6) dIle:Lys ratio 0.76	3.161	2.450	75.84	0.852	26.98	0.033	1.03
7) dIle:Lys ratio 0.83	3.162	2.391	75.56	0.852	26.93	0.030	0.94
8) Positive control (PC)[3]	3.160	2.392	75.70	0.873	27.57	0.026	0.81
SEM[4]	0.037	0.026	0.29	0.017	0.46	0.002	0.05
Regression analysis				Probabilities			
Linear	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003	0.35
Quadratic	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.005	0.52
Contrast							
PC vs. Trt 5	0.64	0.83	0.61	0.60	0.98	0.07	0.07
				Coefficient of determination			
R^2 linear	0.635	0.684	0.206	0.685	0.404	0.136	0.002
R^2 quadratic	0.716	0.771	0.237	0.789	0.494	0.153	0.012

[1]Values are least-square means of 8 replicate pens, with 14 birds being selected and processed at d 42. Experiment 2.
[2]Treatments 1 to 7 are represented by analyzed digestible Ile to Lys ratios.
[3]Calculated digestible Ile to Lys ratio = 0.70.
[4]Pooled standard error.

the incidence of mortality of broilers. The contrast displayed a difference between broilers consuming the PC and Trt 5 for daily dig Ile intake ($P < 0.001$), with the PC fed birds consuming 136 mg more dig Ile. In addition, there was a numerical decrease ($P = 0.051$) of 6 points in FCR for the PC birds relative to those on Trt 5.

Broilers fed increasing dig Ile to Lys ratios displayed positive linear and quadratic effects for carcass weight and yield ($P < 0.001$) (Table 9). Likewise, BMW and BMY responses of broilers increased linearly and quadratically ($P < 0.001$) as dig Ile to Lys ratio increased in the diet from 1.6 to 3.1 kg BW. There was no effect of increasing dig Ile to Lys ratios on abdominal fat yield ($P = 0.52$); however, positive linear and quadratic effects were observed ($P = 0.003$) for abdominal fat weight as dietary dig Ile to Lys ratios were elevated. The contrast did not display any differences between birds fed the PC and Trt 5 for the carcass characteristic of broilers ($P > 0.05$). Broilers fed progressive additions of dig Ile led to an optimum dig Ile to Lys ratio of 0.67 for BWG ($P < 0.001$) from 1.5 to 3.1 kg BW. Similarly, optimum dig Ile to Lys ratios were determined to be 0.67, 0.66, and 0.68 for FCR, BMW, and BMY, respectively, based on linear broken-line regression.

In agreement, Kidd et al. (2004) reported the total Ile requirement to range from 0.64 to 0.69% for growth performance and carcass characteristics from 30 to 42 d of age with a total Lys concentration of 1.05%. These authors also determined that optimum performance for broilers from 30 to 42 d of age could be obtained with a total daily Ile intake of 1,100 mg. This is considerably lower than the values of 1,600 to 1,650 mg/d of dig Ile obtained from broilers in the present research. This indicates that modern broiler strains may have a greater dietary need for Ile to optimize growth relative to genetic

strains of the past. There was an increase of abdominal fat weight as dig Ile to Lys ratios were elevated, however, there was no effect on the fat yield observed. This result is in agreement with what was reported for broilers from 22 to 42 d of age, where dietary Ile concentration affected abdominal fat weight, but did not change yield (Mejia et al., 2011; Miranda et al., 2015). Birds fed Trt 5 had a reduced daily dig Ile intake compared with the broilers provided the PC diet; however, this was not caused by a reduction in FI. This may be due to Trt 5 containing a lower dig Ile concentration compared with the calculated values and was not similar in dig Ile with the PC diet. A numerical decrease was observed for the birds provided the PC diet compared with broilers consuming Trt 5 in FCR, and though it was not a significant effect there was an improvement of 6 point of FCR in the absence of blood cells.

Estimated optimum dig Ile to Lys ratios ranged from a 0.66 to 0.68 for BWG, FCR, BMW, and BMY. These estimates are congruent with previously reported optimum ratios that range from 0.66 to 0.69 for broilers from 1.0 to 3.0 kg BW (Baker, 1996; Mack et al., 1999; Miranda et al., 2015), though dietary dig Lys concentrations varied between studies. Mejia et al. (2011) reported higher optimum ratios of 0.69 for growth performance and 0.72 for BMY while utilizing a similar high producing broiler strain as the present study. Estimated optimum ratios were obtained utilizing linear broken-line regression analysis, as quadratic analysis did not produce a good fit with the response criteria (BWG SEM = 0.029 vs. 0.092). The treatment design was susceptible to being affected by lower analyzed ratios shifting data below the break point of the quadratic broken-line model, preventing a good fit. This was addressed in the experimental designs of Exp 1 and 3 with the addition of an 8th

Table 10. Growth performance of male Yield Plus × Ross 708 broilers fed varying digestible Ile to Lys ratios from 2.6 to 3.9 kg BW (35 to 49 d of age)[1].

Dietary treatments[2]	BW (kg)	BWG[3] (kg)	FI[4] (kg)	Dig Ile Intake[5] (mg/d)	FCR[6] (kg/kg)	Mortality (%)
1) dIle:Lys ratio 0.45	3.621	0.988	2.177	693	2.214	2.55
2) dIle:Lys ratio 0.53	3.744	1.068	2.324	777	2.192	1.15
3) dIle:Lys ratio 0.56	3.850	1.219	2.422	923	1.988	6.25
4) dIle:Lys ratio 0.60	3.882	1.221	2.408	971	1.979	2.20
5) dIle:Lys ratio 0.66	3.921	1.268	2.406	1,051	1.902	4.80
6) dIle:Lys ratio 0.70	3.931	1.311	2.393	1,136	1.832	3.85
7) dIle:Lys ratio 0.72	3.980	1.308	2.426	1,215	1.857	1.40
8) dIle:Lys ratio 0.74	3.900	1.222	2.232	1,119	1.831	1.50
9) Positive control (PC)[7]	3.892	1.261	2.370	1,150	1.882	4.25
SEM[8]	0.034	0.028	0.030	12.15	0.044	0.045
Regression analysis				Probabilities		
Linear	0.001	0.001	0.15	0.001	0.001	0.60
Quadratic	0.001	0.001	0.001	0.001	0.001	0.21
Contrast						
PC vs. Trt 5	0.54	0.85	0.40	0.001	0.75	0.82
				Coefficient of determination		
R^2 linear	0.429	0.426	0.033	0.864	0.503	0.004
R^2 quadratic	0.574	0.629	0.459	0.928	0.523	0.051

[1]Values represent least-square means for 8 replicate pens with 30 chicks per pen at 49 d of age. Experiment 3.
[2]Treatments 1 to 8 are represented by analyzed digestible Ile to Lys ratios.
[3]Body weight gain.
[4]Feed intake.
[5]Determined using digestible Ile concentration of each treatment.
[6]Feed conversion ratio corrected for mortality.
[7]Calculated digestible Ile to Lys ratio = 0.68.
[8]Pooled standard error.

titrated diet, allowing more data above the hypothesized optimum ratio. These data indicate an optimal dig Ile to Lys ratio of 0.66 to 0.68 is appropriate for broilers from 1.6 to 3.1 kg BW.

Experiment 3

Positive linear and quadratic effects were observed for BW and BWG ($P < 0.001$) of broilers as dig Ile to Lys ratios increased in the diet fed from 2.6 to 3.9 kg BW (Table 10). Similarly, dig Ile intake displayed linear and quadratic responses ($P < 0.001$), where values increased linearly and quadratically from Trt 1 to 8. For FI of broilers, quadratic responses were observed ($P < 0.001$), however, there was not a linear relationship between FI and dig Ile to Lys ratio ($P = 0.15$). Broilers that were fed progressive additions of dig Ile displayed decreasing linear and quadratic responses ($P < 0.001$) for FCR. There was no effect of increasing dig Ile to Lys ratios on the mortality of broilers. A difference was elucidated between birds provided the PC and Trt 5 for the daily dig Ile intake of broilers ($P < 0.001$), with the PC producing a larger dig Ile intake response. Blood cell inclusion had no effect on any other growth performance parameters of broilers evaluated from 2.6 to 3.9 kg BW ($P > 0.05$).

Broilers being fed increasing dig Ile to Lys ratios displayed linear and quadratic responses ($P < 0.001$) for carcass weight from 2.6 to 3.9 kg BW (Table 11). However, no effect of dig Ile to Lys ratios on carcass yield was observed ($P = 0.30$) as dig Ile increased in the diet. Positive linear and quadratic effects were observed ($P < 0.001$) for BMW and BMY of broilers when dietary Ile

concentrations were increased. There was no effect of dig Ile to Lys ratios observed for abdominal fat weights ($P = 0.85$) or yields ($P = 0.12$). The contrast may have revealed an effect of blood cells inclusion for both carcass yield ($P = 0.016$) and BMY ($P = 0.017$) between broilers fed Trt 5 and the PC. No other differences were observed between these diets for carcass characteristics ($P > 0.05$). The optimum dig Ile to Lys ratio for BWG was determined at 0.63 ($P < 0.001$) utilizing linear broken-line regression from 2.6 to 3.9 kg BW (Table 6). Similarly, the optimum dig Ile to Lys ratio for FCR was determined as 0.69 ($P < 0.001$). The optimum dig Ile to Lys ratios for BMW and BMY were observed at 0.69 and 0.73 ($P < 0.001$), respectively, with linear broken-line analysis.

Kidd et al. (2004) reported the total Ile requirement of broilers from 42 to 56 d of age to range from 0.57 to 0.67% with a dietary Lys concentration of 0.90%. These authors reported daily total Ile intake necessary to obtain optimum performance of 1,154 mg, which agrees with the range of 1,100 to 1,200 mg/d that was obtained in the current study, though these are dig values. However, broilers from the previous study were grown approximately 400 g larger than birds from Exp 3. Therefore, evidence suggests that modern broiler strains may require more dietary Ile per unit of growth compared with genetic strains of the past. The daily dig Ile intakes of birds fed the PC were higher compared with Trt 5 broilers; however, there was no effect observed for FI. This is likely caused by Trt 5 containing an analyzed dig Ile to Lys ratio of 0.66, rather than the calculated ratio of 0.68. Increases in carcass and breast meat yields were also observed for birds consuming the PC compared with Trt 5. This response may be due to Trt 5

Table 11. Carcass characteristics of male Yield Plus × Ross 708 broilers fed diets with increasing digestible Ile to Lys ratios from 2.6 to 3.9 kg BW (35 to 49 d of age)[1].

Response parameter	Live Weight, kg	Carcass		Breast meat		Abdominal fat	
		Weight, kg	Yield, %	Weight, kg	Yield, %	Weight, kg	Percentage, %
Dietary treatments[2]							
1) dIle:Lys ratio 0.45	3.678	2.801	76.28	0.987	26.82	0.034	0.92
2) dIle:Lys ratio 0.53	3.777	2.879	76.25	1.017	26.95	0.037	0.97
3) dIle:Lys ratio 0.56	3.937	3.014	76.53	1.075	27.28	0.035	0.88
4) dIle:Lys ratio 0.60	3.950	3.008	76.15	1.076	27.24	0.035	0.89
5) dIle:Lys ratio 0.66	3.967	3.031	76.37	1.086	27.39	0.033	0.82
6) dIle:Lys ratio 0.70	4.026	3.085	76.71	1.122	27.81	0.032	0.81
7) dIle:Lys ratio 0.72	4.045	3.101	76.84	1.120	27.72	0.034	0.85
8) dIle:Lys ratio 0.74	3.991	3.050	76.46	1.108	27.77	0.036	0.90
9) Positive control (PC)[3]	3.970	3.065	77.29	1.113	28.10	0.033	0.82
SEM[4]	0.038	0.030	0.27	0.014	0.22	0.002	0.05
Regression analysis				*Probabilities*			
Linear	< 0.001	< 0.001	0.12	< 0.001	< 0.001	0.85	0.12
Quadratic	< 0.001	< 0.001	0.30	< 0.001	< 0.001	0.82	0.12
Contrast							
PC vs. Trt 5	0.95	0.42	0.016	0.13	0.017	0.98	0.99
				Coefficient of determination			
R^2 linear	0.447	0.447	0.037	0.507	0.283	0.001	0.039
R^2 quadratic	0.567	0.560	0.039	0.584	0.288	0.007	0.066

[1]Values are least-square means of 8 replicate pens, with 14 birds being selected and processed at d 50. Experiment 3.
[2]Treatments 1 to 8 are represented by analyzed digestible Ile to Lys ratios.
[3]Calculated digestible Ile to Lys Ratio = 0.68.
[4]Pooled standard error.

having a lower dig Ile concentration than it was formulated to contain.

Optimum dig Ile to Lys ratios ranged from 0.69 to 0.73 for FCR, BMW, and BMY. Body weight gain produced an optimum ratio of 0.63, which is lower than previously reported ratios for this time period of 0.68 and 0.69 (Baker, 1996, 1997). The estimated optimum ratios for FCR and BMW were at 0.69, which is in agreement with previous reports for broilers grown to approximately 3.5 kg BW (Baker, 1997). The difference observed between responses of BWG and FCR have been observed previously for broilers of >3.0 kg (Kidd et al., 2000, 2004). These authors reported that the difference can be rationalized by increased FI responses to compensate for the limiting nutrient. Breast meat yield produced an optimum dig Ile to Lys ratio of 0.73, which is higher than previously published data (Corzo et al., 2002, 2008); however, it is in agreement with the dig Ile ratio obtained by Mejia et al. (2011) of 0.72 for broilers from 28 to 42 d of age. Based on these data, an optimal dig Ile to Lys ratio of 0.69 to 0.70 is appropriate for broilers from 2.5 to 3.9 kg BW.

Statistical Models

Consideration must be given to the statistical model chosen to evaluate optimum dig AA ratios, as it can affect the results obtained. Broken-line regression analysis is commonly used as it provides a function that describes the responses to nutrient doses across all concentrations and provides a break point estimate of the optimum with an associated standard error (Robbins et al., 2006). However, the type of broken-line model fit to a given data set can change the output of the analysis

based on the shape of the data. Linear broken-line regression presumes that the responses to a nutrient dose are linear, when in most cases of dose response designs responses are curvilinear in nature (Robbins et al., 2006). In these cases, linear broken-line analysis can still provide a satisfactory fit to the data; however, this model can underestimate a requirement compared to a quadratic model that achieves a significant fit. The issue that is observed with quadratic broken-line models is that a minimum of 3, and preferably 4, data points are required above the break point of the model for it to accurately predict an optimum requirement (Robbins et al., 2006). These authors reported that problems can also be observed if there are large variations in the responses of broilers above a hypothesized optimum that affect the shape of the plateau. Many of these issues occurred in the present research, and likely affected the ability of the quadratic broken-line model to accurately predict the optimum dig Ile to Lys ratios of broilers. Additionally, Ile concentrations being analyzed lower than formulated values may have contributed to a linear broken-line model better fitting the data in many cases. The lower values shifted the data points down so that there were insufficient data above the break point for a quadratic model to estimate the optimums. Based on the responses observed, both linear and quadratic broken-line analysis provided accurate estimates of dig Ile ratios from 1.0 to 4.0 kg BW dependent upon the trial and variable (Table 7).

Response Criteria

In the current research, optimum dig Ile to Lys ratios varied based on the response criteria. Optimum dig Ile

to Lys ratios are more pronounced with breast meat yield compared with growth performance (Kidd et al., 2004; Mejia et al., 2011; Miranda et al., 2015). This response was observed for Exp 2 and 3, with optimum ratios for BMY being 3 to 5 points greater than growth performance characteristics. Experiment 1 produced a higher optimum ratio for BMW relative to other parameters, including BMY. Corzo et al. (2002) reported that Ile needs of broilers heavier than 3.0 kg are greater for breast tissue development relative to other growth. However, similar responses have been reported for broilers from 1.5 to 2.5 kg as well (Kidd et al., 2004). This is critical, especially if final market weights larger than 3.0 kg are being targeted. Feeding higher dig Ile to Lys ratios through the grower and finisher periods may help to optimize breast meat yields.

In conclusion, data from these 3 experiments indicated that the optimum dig Ile to Lys ratios for growth performance are largely in agreement with previous research. However, the Ile intake required to optimized growth and feed efficiency may be greater in modern broiler strains relative to those of the past. Estimated optimums for BMY in the current studies are greater than previously reported for broilers and may also warrant increased Ile intake. Corn and soybean meal-based diets formulated with a dig Ile to Lys ratio of 0.67 to 0.69 will be adequate for broilers to obtain optimum growth performance and optimum meat yield responses from 1.0 to 4.0 kg BW.

DISCLOSURES

REFERENCES

Association of Official Analytical Chemists International. 2006. Official Methods of Analysis (18th ed.). Association of Official Analytical Chemists, Arlington, VA.

Bae, S. H., J. H. Kim, I. S. Shin, and K. Han. 1999. Partition of amino acid requirements of broilers between maintenance and growth. V. isoleucine and valine. Asian Australas. J. Anim. Sci. 12:388–394.

Baker, D. H. 1996. Advances in amino acid nutrition and metabolism of swine and poultry. Pages 41−54 in Nutrient Management of Food Animals to Enhance and Protect the Environment. E. T. Kornegay, ed. CRC Press Inc, Boca Raton, FL.

Baker, D. H. 1997. Ideal amino acid profiles for swine and poultry and their applications in feed formulation. BioKyowa Technical Review 9:154–178.

Baker, D. H. 2003. Ideal amino acid patterns for broiler chicks. *Amino Acids in Animal Nutrition* (2nd ed). CAB International, Oxon, United Kingdom, 223–236.

Baker, D. H., A. B. Batal, T. M. Parr, N. R. Augspurger, and C. M. Parsons. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81:485–494.

Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. Poult Sci 74:1441–1447.

Barbour, G., and J. D. Latshaw. 1992. Isoleucine requirement of broiler chicks as affected by the concentrations of leucine and valine in practical diets. Br. Poult. Sci. 33:561–568.

Bender, D. A. 2012. The Branched-Chain Amino Acids: Leucine, Isoleucine and Valine. *Amino Acid Metabolism* (3rd ed). John Wiley & Sons, Ltd, Hoboken, NJ, 279–302.

Berres, J., S. L. Viera, M. T. Kidd, D. Taschetto, D. M. Freitas, R. Barros, and E. T. Nogueira. 2010. Supplementing L-valine and L-isoleucine in low-protein corn and soybean meal all-vegetable diets for broilers. J. Appl. Poult. Res. 19:373–379.

Corzo, A., W. A. Dozier III, M. T. Kidd, and D. Hoeler. 2008. Impact of dietary isoleucine status on heavy-broiler production. Int. J. Poult. Sci. 7:526–529.

Corzo, A., R. E. Loar II, and M. T. Kidd. 2009. Limitations of dietary isoleucine and valine in broiler chick diets. Poult. Sci. 88:1934–1938.

Corzo, A., E. T. Moran, and D. Hoehler Jr.. 2002. Isoleucine needs of broiler males from 42 to 56 days of age. Poult. Sci. 80:25 Abstr.

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715–724.

Dozier, W. A. III, P. B. Tillman, and J. Usry. 2012. Interactive effects of digestible valine-and isoleucine-to-lysine ratios provided to male broilers from 4 to 6 weeks of age. J. Appl. Poult. Res. 21:838–848.

Farran, M. T., and O. P. Thomas. 1990. Dietary requirements of leucine, isoleucine, and valine in male broilers during the starter period. Poult. Sci. 69:757–762.

Garcia-neto, M., Perri, S. H. V. 2015. PPM: Programa prático de modelagem. Disponivel em. Accessed June 2020. https://sites.google.com/site/programapraticodemodelagem/

Hale, L. L., G. T. Pharr, S. C. Burgess, A. Corzo, and M. T. Kidd. 2004. Isoleucine needs of thirty- to forty-day-old female chickens: immunity. Poult. Sci. 83:1979–1985.

Kidd, M. T., D. J. Burnham, and B. J. Kerr. 2004. Dietary isoleucine responses in male broiler chickens. Br. Poult. Sci. 45:67–75.

Kidd, M. T., B. J. Kerr, J. P. Allard, S. K. Rao, and J. T. Halley. 2000. Limiting amino acid responses in commercial broilers. J. Appl. Poult. Res. 9:223–233.

Mack, S., D. Bercovici, G. De Groote, B. Leclercq, M. Lippens, M. Pack, J. B. Schutte, and S. Van Cauwenberghe. 1999. Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. Br. Poult. Sci. 40:257–265.

Mejia, L., C. D. Zumwalt, E. J. Kim, P. B. Tillman, and A. Corzo. 2011. Digestible isoleucine to-lysine ratio effects in diets for broilers from 4 to 6 weeks posthatch. J. Appl. Poult. Res. 20:485–490.

Miranda, D. J. A., S. L. Vieira, A. Favero, C. R. Angel, C. Stefanello, and E. T. Nogueira. 2015. Performance and meat production of broiler chickens fed diets formulated at different crude protein levels supplemented or not with L-valine and L-isoleucine. Anim. Feed Sci. Tech. 206:39–47.

National Research Council. 1994. Nutrient Requirements of Poultry (9th rev. ed). National Academy Press, Washington, DC.

Robbins, K. R., A. M. Saxton, and L. L. Southern. 2006. Estimation of nutrient requirements using broken-line regression analysis. J. Anim. Sci. 84(E. Suppl.):E155–E165.

Rostagno, H. S. 2017. Brazilian Tables for Poultry and Swine, Composition of Feedstuffs and Nutrient Requirements (4th ed). Federal University of Vicosa, Minas Gerais, Brazil.

SAS Institute. 2017. SAS® User's Guide. (Version 9.4 ed). SAS Institute Inc., Cary, NC.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Tech. 59:215–221.