Determination of the optimal dietary isoleucine-to-lysine ratio in high yielding broilers during the starter phase

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Abstract

Continued efforts to realize the benefits of decrease dietary crude protein, the recent availability of isoleucine (Ile) in synthetic form, and rapid improvements in genetics for high yielding broiler strains require the re-evaluation of the optimal dIle:dLys in broilers in order to maximize performance. There is limited research published on optimal dIle:dLys during the early growth phase of broilers. Thus, this study was designed to evaluate the dIle requirement of Ross 708 x Ross YP male broilers from 0-18 days of age. Seven experimental diets (ratios of dIle:dLys = 52, 57, 62, 67, 72, 77, and 82%) were created by blending a common deficient corn and soybean meal-based diet with a dIle:dLys of 52% and a summit diet containing dIle:dLys of 82%. A practical control diet containing 67% dIle:dLys was manufactured separately to verify the blended control diet (50 deficient:50 summit blend; 67% dIle:dLys). Eight experimental treatments were fed to 12 replicate pens of 25 birds from 0-18 days of age. Body weight gain (BWG) and Feed Conversion Ratio (FCR) were measured at 0 and 18d. dIle:dLys ratios were estimated using quadratic regression (QR; 95% of the asymptote), as well as linear and quadratic broken line models (LBL; QBL). The performance result of the experiment demonstrates that feeding higher than starter breeder's recommendations (67% dIle:dLys) at the time of this study improved broiler performance from 0-18 d. Regression analyses demonstrated the optimum dIle:dLys ratios varied from 63 to 73% for BWG and 68-74% for FCR using the three different statistical methodologies (LBL, QBL, and QR). Understanding the optimal dietary ratios of dIle in the diet will help nutritionists formulate diets according to ideal protein concept to maximize broiler performance, lower the CP levels in the diets, lower feed cost. This will maximize nitrogen efficiency and retention, resulting in lower nitrogen excretion, improved litter conditions, enhanced bird welfare, all of which result in sustainable and profitable production.

Background

Formulating diets following the concept of ideal protein ratio relative to Lysine (Lys) allows for the most efficient utilization of crude protein in the diet by maximizing nitrogen efficiency/retention while minimizing nitrogen excretion (Emmert and Baker, 1997). Commercial availability of feed grade amino acids at an economically feasible price has made it easier for a nutritionist to consider adding 4th, 5th, and 6th limiting amino acids in the formulation. This has helped to reduce crude protein and minimize excess amino acid nutrient matrix, diet cost, improve production, welfare, and environmental factors, with a goal towards more sustainable poultry production (van Harn et al., 2019; Chrystal et al., 2020). Such adoption has no detrimental effects on animal performance as long as the indispensable amino acid requirements/ratios for the birds are met during formulation (Kidd et al., 2004; Selle et al., 2020).

Ile can be 4th, 5th, or 6th limiting or co-limiting amino acid in broilers in the diet formulation depending on the ingredients used. The optimal ratio of dIle:dLys in the diet may affect the metabolic efficiency and growth performance of the bird and the utilization of Valine (Val), Leucine (Leu), and other limiting amino acids (Corzo et al., 2009). Along with Val and Leu, Ile is one of the branch chain amino acids(BCAA), if unbalanced, can cause negative effects on performance. Thus, it is even more critical to understand and meet the Ile requirement in the diet. In the past when feed grade Ile was not available at an economically feasible price, Ile was either spec'd to meet/exceed requirement by macro ingredients resulting in an imbalanced matrix of other amino acids or ignored to be limited in practical diets. With the availability of L-Ile, there is an opportunity to provide the precise amount of Ile in the diet for feed cost savings and/or reduce CP in the diet and maximize performance.

The Ile requirement for older broilers has been widely researched for multiple decades (Velu et al., 1972; Burnham and Gous, 1992; Baker et al., 2002; Hale et al., 2004; Campos et al., 2009; Corzo et al., 2009; Helmbrecht et al., 2010). However, due to the continuous improvement of broiler genetics, the starter growth phase represents an increasing proportion of a broiler's lifecycle. Within this critical time period, maximal body growth is accomplished by meeting the bird's high demand for energy and protein (Behnke and Beyer, 2002; Willemsen et al. 2008). However, few reports have addressed the optimal dIle:dLys ratio during the starter phase, especially including when the first week of the broiler's life. The latest reports published on the Ile requirement for broiler utilized Cobb birds from 7-21 days of age by Helmbrecht et al. (2010) and Campos et al., (2012). Research studies elucidating the Ile needs of male Ross 708 birds during the early phase are sparse and verifies the need for current industry relevant data. Thus, this study was designed to understand the optimal ratio of Ile of male Ross 708 x Ross YP male broilers from 0-18 days of age.

Methods

All experimental procedures were approved by Mississippi State University Institutional Animal Care and Use Committee guidelines, and birds were raised in adhering to the Care and Use of Agricultural Animals in Research and Teaching. A total of seven experimental pelleted/crumbled diets were created from a common deficient corn and soybean meal-based diet providing a dIle:dLys of 52% (Table 1). After a deficient diet was created; one-half was retained for the creation of the summit diet (82% dIle:dLys). To provide an additional five diets varying in dIle:dLys, the Deficient and Summit diets were blended in different ratios prior to pelleting to create the remaining dIle:dLys ratios of 57, 62, 67 (BLEND-CON), 72, and 77%, respectively. A practical control diet (PRAC-CON; 67% dIle:dLys) (Table 1) was manufactured separately for verification of the blended control diet (50 deficient:50 summit blend; BLEND-CON; 67% dIle:dLys). Prior to formulation, corn and soybean meal were analyzed for total AA content (AOAC 982.30 mod, 994.12 mod, 988.15 mod; Eurofins Scientific Inc., Des Moines, IA), as well as scanned using Near Infrared (NIR) Spectroscopy (FOSS) to obtain digestibility coefficient values for more accurate formulation.

Table 1. Experimental diet formulations for the deficient and PRAC – CON[1] diets during the starter phase (d 0-18)

Ingredient Name	52% dIle:dLys (Deficient)	67% dIle:dLys (PRAC – CON)
Corn	68.34	58.09
Soybean meal (48% CP)	25.07	35.83
Poultry fat	0.92	2.92
L-Glutamine	0.79	-
Glycine	0.49	-
Defluorinated phosphate	1.61	1.57
Calcium carbonate	0.41	0.33
Sodium Bicarbonate	0.14	0
Salt	0.11	0.27
Copper Sulfate	0.05	0.05
L-Lysine HCl	0.47	0.12
L-Threonine	0.27	0.13
L-Valine	0.25	0.07
L-Tryptophan	0.02	-
L-Arginine	0.31	-
Phytase[2]	0.02	0.02
L-Methionine	0.42	0.31
Vitamin-trace mineral	0.25	0.25
Choline Cl-60%	0.02	0.02
Coccidiostat[3]	0.05	0.05
Nutrient Name	**Calculated Nutrients (%)**	
AME (kcal/kg)	3000	3000
Crude protein (%)	20.55	22.13
Calcium (%)	0.96	0.96
Total phosphorus (%)	0.62	0.66
Available phosphorus (%)	0.48	0.48
Sodium (%)	0.16	0.18
Potassium (%)	0.74	0.93
Chloride (%)	0.19	0.22
Digestible Lysine (%)	1.22	1.22
Digestible Methionine (%)	0.69	0.63
Digestible Methionine + Digestible Cysteine (%)	0.91	0.9
Digestible Tryptophan (%)	0.21	0.25
Digestible Threonine (%)	0.82	0.82
Digestible Isoleucine (%)	0.63	0.82
Digestible Valine (%)	0.92	0.91
Digestible Arginine (%)	1.13	1.32

[1] 67% dIle:dLys (PRAC-CON), was made to compare to the blended diet 67% dIle:dLys BLEND-CON).
Deficient (52% dIle:dLys) and Summit (82% dIle:dLys) basal diets were batched and mixed in different ratios prior to pelleting for the creation of the remaining dIle:dLys ratios and can be found below.
- 52% dIle:dLys – 100:0 (Deficient:Summit)
- 57% dIle:dLys– 83.4:16.7 (Deficient:Summit)
- 62% dIle:dLys– 66.7:33.3 (Deficient:Summit)
- 67% dIle:dLys– 50:50 (Deficient:Summit)
- 72% dIle:dLys– 33.3:66.7 (Deficient:Summit)
- 77% dIle:dLys– 16.7:83.3 (Deficient:Summit)
- 82% dIle:dLys– 0:100 (Deficient:Summit)

[2] *E.Coli* phytase; sparing 0.15% Ca and aP

[3] Zoamix (Zoalene; 0.0125% inclusion). Zoetis, Parsippany, NJ.

Eight dietary treatments were allocated to 96 floor pens in a complete randomized block design. On the day of hatch, a total of 2,400 male Ross 708 x Ross YP male broiler chicks were equally allocated to 96 pens to achieve 25 birds per pen. Each pen was considered an experimental unit. Each pen was weighed on d 0 and 18. Average body weight (BW), BW gain (BWG), average feed intake/bird (FI), feed conversion ratio (FCR) adjusted for mortality from d 0 to 18, and total Ile intake/bird (g) were calculated.

Performance data were analyzed using the GLM procedure in SAS (SAS Institute Inc., Cary, NC); differences among means were explored by Fisher's least significant difference, and statistical significance was set at P-value ≤ 0.05. Preplanned contrasts were also performed to compare between the PRAC-CON (67% dIle:dLys) and the BLEND-CON (67% dIle:dLys). dIle:dLys requirement for BWG and FCR was estimated utilizing multiple methods. First, data were analyzed using PROC REG (SAS Institute Inc., Cary, NC). Methods employed were a linear regression model and then a quadratic regression model (QR), in which the dIle requirement was calculated at 95% of the asymptote when a significant quadratic response was observed (P ≤ 0.05). Next, linear broken line (LBL) and quadratic broken line (QBL) models were determined when a significant response occurred (P ≤ 0.05) using Practical Program for Modeling (Goncalves et al., 2015) and then confirmed using the PROC NLIN option of SAS (SAS Institute Inc., Cary, NC; Robbins et al., 2006). Different methods and each model were considered in this experiment as each method gives insight to a variety of questions while determining nutritional requirements (Pesti et al., 2009).

Results and Discussion:

Data from this experiment indicate that broilers fed 52% dIle:dLys had the lowest BW and BWG (P < 0.0001) at 18 days of age. Birds fed 77 and 82% dIle:dLys demonstrated the highest BW and BWG; while 62, 67 (BLEND-CON), and 72% dIle:dLys performed statistically similar (Table 2). Preplanned contrasts demonstrated no statistical differences when individually comparing 67% dIle:dLys (PRAC-CON) to 67 (BLEND-CON) dIle:dLys (P = 0.257; Table 2). Lower level of Ile in the diets caused depression in BWG and increases FCR compared to higher levels of Ile in the diet, which was in agreement with previous publications, (Kidd et al., 2000; Kidd et al., 2004; Hale et al., 2004; Maynard et al., 2021).

Table 2. The effect of varying % dIle:dLys concentrations on day 0 to 18 Ross 708 x Ross YP male broiler performance

% dIle:dLys Ratios	d0 Avg[1]BW[2] (kg)	d18 Avg[1] BW[2] (kg)	BWG[3] (kg/bird)	FI[4]/bird (kg)	FCR[6]
52	0.043	0.626[d]	0.583[d]	0.795	1.300
57	0.043	0.665[c]	0.621[c]	0.771	1.295
62	0.043	0.685[ab]	0.642[ab]	0.806	1.245
67 (BLEND-CON)[8]	0.043	0.680[bc]	0.637[bc]	0.792	1.257
72	0.043	0.691[ab]	0.647[ab]	0.814	1.235
77	0.043	0.698[a]	0.655[ab]	0.813	1.242
82	0.043	0.700[a]	0.657[a]	0.822	1.245
67 (PRAC-CON)[8]	0.043	0.698[a]	0.655[ab]	0.818	1.237
P-value	0.611	<.0001	<.0001	0.277	0.059
SEM[9]	0.00004	0.007	0.007	0.015	0.018

Differing letters within a column demonstrate a significant difference
[1] Average
[2] Body Weight
[3] Body Weight Gain
[4] Feed Intake is based on a per bird basis
[5] Mortality percentage is based on a beginning pen number of birds per pen (25)
[6] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[7] Total isoleucine intake g/bird was calculated utilizing the analyzed total isoleucine of the diet (Table 2) fed during the feeding period and multiplying it by the intake during the feeding period on a per bird basis.
[8] The BLEND-CON diet was formulated to ratio of 67% digestible Isoleucine:digestible Lysine (dIle:dLys). In order to verify the mixing technique, The PRAC-CON was made and compared to the BLEND-CON which was the formulated to contain 67% dIle:dLys
[9] Standard Error of the Mean

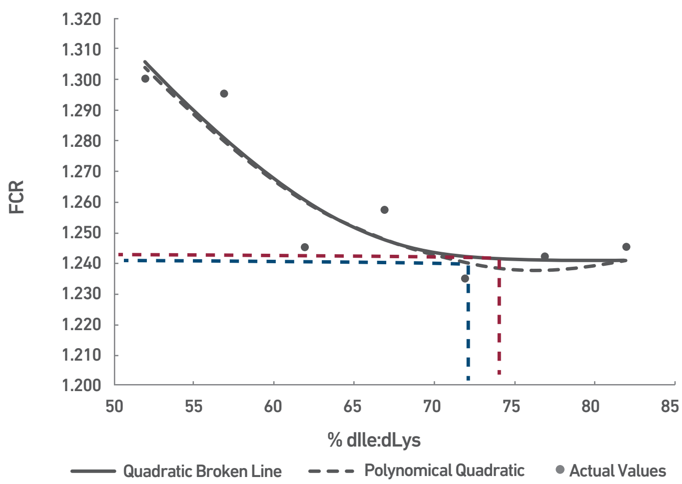
Significant QR, LBL, and QBL responses were observed for increasing dietary dIle:dLys levels for BWG from 0 to 18 d (P < 0.05; Table 3). These data suggest that the dIle:dLys requirement for Ross 708 × Ross YP male broilers for BWG was 73% based on QR (P = 0.006; R2 = 0.80); 63% based on LBL (P = 0.005; R2 = 0.93); and 66% based on QBL (P = 0.004; R2 = 0.94). Similarly, the ratio for dIle:dLys for FCR, was 72% based on QR (P = 0.023; R2 = 0.85); 68% based on LBL (P = 0.027; R2 = 0.86); and 74% based on QBL model (P = 0.020; R2 = 0.90) (Table 3; Figure 1). The requirements demonstrate differences when utilizing different statistical models; however, these differences were expected (Pesti et al., 2009). The data suggest the dIle:dLys requirement for Ross 708 × Ross YP male broilers during the first 18 days of age ranges from 63-73% for BWG, and 68-74% for FCR. Similarly, previous research on the dIle:dLys requirements

utilizing different commercial broiler strains, sexes, and experimental period have concluded the requirement for FCR to be higher than for BWG (Kidd et al., 2000; Corzo et al., 2004; Kidd et al., 2004; Campos et al., 2009; Dozier et al., 2012; Tillman and Dozier, 2013). Campos et al. (2012) examined the dIle:dLys ratio in Cobb birds at 7-21d of age with three points on the curve (60, 65, 70) which showed linear responses for BWG and FCR. In the study conducted with Cobb birds from d 7-21d of age, utilizing 95% of Qmax and QBL estimated the dIle:dLys to be 64-69% for BW, BWG, and FCR; while using LBL, the dIle:dLys requirements were 61% for BWG and FCR (Helmbrecht et al., 2010; Tavernari et al., 2012; Tillman and Dozier, 2013).
This requirement only represents the biological responses of amino acids in the birds. Due to the opportunity for greater potential economic return, cost of poultry meat, and rapidly changing cost and availability of feed ingredients, nutritionists have embraced amino acid levels that optimize broiler performance but also maximize economic return (Pesti et al., 2009; Kidd and Tillman, 2016). Previous research has supported that the amino acid level that improved broiler performance (BW, BWG, and FCR) also maximized economic return (Perryman et al., 2013; Tillman et al., 2013).

Table 3. Digestible Isoleucine: digestible Lysine (% dIle:dLys) requirements of Ross 708 × Ross YP male broilers from 0 to 18 days of age based on linear broken line, quadratic broken line, and quadratic regression models

Model	Response variable	Estimated % dIle:dLys requirement	Estimated % dIle requirement [1]	P-value	R^2
Linear broken line	BWG[3]	63	0.76	0.005	0.93
	FCR[4]	68	0.83	0.027	0.86
Quadratic broken line	BWG	66	0.8	0.004	0.94
	FCR	74	0.9	0.02	0.85
Quadratic Regression[2]	BWG[5]	73	0.89	0.006	0.8
	FCR[6]	72	0.88	0.023	0.85

[1] Estimated % dIle based off 1.22 dLys
[2] Quadratic Regression model, in which the dLys requirement was calculated by 95% of the asymptote
[3] Body Weight Gain (kg)
[4] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight
[5] Body Weight Gain. Calculated values were derived using the regression equation: $y = -0.65034 x^2 + 1.22476 x + 0.07887$; where y = Body Weight Gain and x = dIle
[6] Feed Conversion Ratio (Feed:Gain) was adjusted with mortality weight. Calculated values were derived using the regression equation: $y = 0.73595 x^2 + -1.36756 x + 1.87331$; where y = FCR and x = dIle



Quadratic Broken line
P = 0.020
SSE = 0.0006
R^2 = 0.85
Polynomial Quadratic
P = 0.023
R^2 = 0.85
[1]Feed Conversion Ratio;

Figure 1. Quadratic Broken Line and Polynomial Quadratic Analysis for d 0-18 FCR[1] for Ross 708 x Ross YP male broilers

Conclusion

The current study demonstrated that the optimum Ile ratio for this male high-yielding broiler exceeded dIle:dLys breeder recommendations at the time of the study (>67%) from 0-18 d of age. The optimum ratio of dIle:dLys varied among statistical models and the response variable. Regression analysis using LBL, QBL, and QR demonstrated the optimum ratio for dIle:Lys ratios for BWG to range from 63-73% and for FCR ranged from 68-74%.

As alternate ingredients are introduced to diet formulation apart from corn and SBM, the Leu level of the diet could increase significantly, leading to increased requirement of Ile and Val in the diet. Understanding the optimum required level of Ile in the diet and formulating accordingly can reduce the potential antagonistic effect among BCAAs, increase production efficiency, and lower diet cost. Knowing optimal Ile ratio for maximum performance and availability of feed grade Ile provides nutritionists a tool for better utilization of lower limiting amino acids to advance towards more sustainable poultry production.

More research needs to be done to understand the optimal requirement of Ile in early diets as we advance into understanding the BCAA interaction, adopt lower crude protein diets utilizing available synthetics amino acids to balance 4th, 5th, and 6th limiting amino acids, and introduce alternate feed ingredients in the diets fed to the rapidly advancing genetics of modern commercial broilers.

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