Ideal Ratio (Relative to Lysine) of Tryptophan, Threonine, Isoleucine, and Valine for Chicks During the Second and Third Weeks Posthatch

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ABSTRACT Six bioassays were conducted to determine the ideal ratios of several amino acids relative to Lys. Young male crossbred chicks were fed diets based on corn gluten meal and synthetic amino acids that could be made singly deficient in Lys, Trp, Thr, Ile, or Val. Diets for all assays contained 3,400 kcal ME/kg, and L-glutamic acid was used to make all diets (within and among assays) equal in crude protein at 22.5% of the diet. True digestibility assessment of corn gluten meal in cecectomized roosters facilitated dose-titration studies so that least squares fitted one-slope broken-lines and quadratic regression equations could be calculated to establish inflection points for weight gain and gain:feed. Four battery pens of four chicks were fed one of six amino acid levels from 8 to 21 or 22 d posthatching. Weight gain and gain:feed responded quadratically ($P < 0.01$) to increasing doses of digestible Lys (0.68 to 1.28%), Trp (0.09 to 0.24%), Thr (0.41 to 0.81%), Ile (0.45 to 0.95%), and Val (0.51 to 1.06%). Broken-line least squares analysis predicted breakpoints for gain and gain:feed, respectively, of: Lys (0.85, 0.96%), Trp (0.16, 0.16%), Thr (0.53, 0.53%), Ile (0.59, 0.58%), and Val (0.74, 0.74%). The intercept of the quadratic regression curve and the plateau of the broken line predicted digestible Lys requirements for gain and gain:feed, respectively, of 0.95 and 1.03%. Similar calculations predicted digestible Trp requirements of 0.18% for gain and gain:feed, digestible Thr requirements of 0.59% for gain and 0.60% for gain:feed, digestible Ile requirements of 0.68% for gain and gain:feed, and digestible Val requirements of 0.81% for gain and 0.82% for gain:feed. Regardless of curve-fitting method, gain:feed requirements for Lys were much higher than weight gain requirements. Using the higher of the broken-line requirement estimates for gain and gain:feed, ideal ratios (% of Lys) were as follows: Lys (100), Trp (16.6), Thr (55.7), Ile (61.4), and Val (77.5).

(*Key words*: broiler, ideal ratio, lysine, amino acid)

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INTRODUCTION

Many factors can influence amino acid (AA) requirements of chicks (and other animals) at any given growth stage (Baker, 1997), i.e., dietary factors (e.g., protein level, energy level, and presence of protease inhibitors), environmental factors (e.g., disease, crowding, feeder space, and heat or cold stress), and genetic factors (e.g., sex and capacity for lean vs. fat growth). Thus AA requirements cannot apply to all birds under all dietary, environmental, and body compositional conditions (Baker and Han, 1994). Pig nutritionists were the first to address these problems by expressing AA requirements as ratios to Lys for different weight categories (Wang and Fuller, 1989; Chung and Baker, 1992a). Although AA requirements change due to the factors mentioned above, the ideal ratios remain similar, and thus only an accurate requirement for Lys needs to be established. The benefit of ideal AA ratios is that once a ratio is established for a certain age period, one can concentrate on accurately determining the Lys requirement under a variety of conditions and then calculate the requirement for all other AA under this condition based on the Lys requirement and ideal ratios (Baker, 1997).

Lysine was chosen as the reference AA because it is used almost exclusively for protein accretion, it is a limiting AA in reduced protein corn-soybean meal broiler diets, and the analysis for Lys is uncomplicated (Baker, 1997). Thus, estimating an accurate Lys requirement is essential because it is the basis for setting requirements for all other indispensable AA. Certain steps need to be taken in order to accurately determine AA ratios to Lys. The same basal diet should be used in determining the requirements for all AA, and all diets within and between bioassays should be isonitrogenous and isoenergetic. Digestible AA values should be used to eliminate differences in absorption efficiencies due to feedstuff sources (Emmert and Baker, 1997). Also, all requirement bioassays should be conducted with the same sex and strain

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Abbreviation Key: AA = amino acid; CGM = corn gluten meal.

of chick as well as during the same growth period. Moreover, when determining AA requirements, the AA doses should encompass the linear and plateau areas of the growth curve.

Mack et al. (1999) used a low protein (17.2% CP) corn-soybean meal diet to evaluate the digestible requirement for several AA, including Lys, Trp, Thr, Ile, and Val. The birds in their bioassays were fed experimental diets during the growth period 20 to 40-d posthatching. However, with regards to Trp and Ile, the weight gain and gain:feed responses were questionable, and this, therefore, did not allow a good basis for establishing Trp and Ile requirements or Trp:Lys and Ile:Lys ratios. Also, Mack et al. (1999) obtained data suggesting that the ideal Thr:Lys ratio was lower (59%) than our previously reported value of 67% (Baker, 1997). It therefore seemed important to obtain more empirical evidence from which ideal amino acid ratios could be calculated.

The objectives of our studies were to 1) establish accurate digestible Lys, Trp, Thr, Ile, and Val requirements under constant environmental, dietary, gender, age, and bird-strain conditions, and 2) use the requirement estimates to establish ideal AA ratios for chicks 8 to 22 d of age.

MATERIALS AND METHODS

General Procedures

All procedures were approved by the University of Illinois Committee on Laboratory Animal Care. Six chick assays were conducted using New Hampshire × Columbian Plymouth Rock male chicks. This broiler strain has been shown to deposit protein as a proportion of weight gain at the same rate as commercial strains, and the Lys requirement (% of diet) is similar as well (Han and Baker, 1991). Chicks were housed in thermostatically controlled starter batteries[2] with raised wire floors in an environmentally controlled building. The chicks were pretested on a fully fortified corn-soybean meal diet containing 23% CP until 1600 h of Day 7 posthatching. On Day 8 posthatching after being subjected to an overnight period of feed withdrawal, chicks were weighed, wing-banded and randomly allotted to pens such that each pen had a similar initial weight and weight distribution. The average initial weight of birds at assay initiation ranged from 90 to 103 g.

Four replicate groups of four chicks were allowed access ad libitum to each experimental diet from 8 to 21 d (Assay 1, 2, 4, 5, and 6) or from 8 to 22 d (Assay 3) posthatching. Body weight of individual chicks within each replicate group, and group feed intakes, were measured at the termination of each experiment. Weight gain and feed efficiency (gain:feed ratio) were then calculated for each pen replicate.

[2]Petersime Incubator Co., Gettysburg, OH 45328.

TABLE 1. Composition (as-fed basis) of the amino acid-deficient basal diets[1,2]

Ingredient	%
Cornstarch	30.871
Dextrose	28.200
Corn gluten meal[3]	18.340
Soybean oil	4.000
Mineral premix[4]	5.370
Sodium bicarbonate ($NaHCO_3$)	0.500
Vitamin premix[5]	0.200
Choline chloride	0.200
L-Glutamic acid	11.000
DL-Met	0.169
L-Arg	0.800
L-Cys	0.187
L-His HCl·H_2O	0.138
Bacitracin MD premix[6]	0.025
DL-α-Tocopheryl acetate (20 mg/kg)	+
Ethoxyquin (125 mg/kg)	+

[1]The diet contained 3,400 kcal ME/kg diet and 12% CP furnished by corn gluten meal (CGM) and was fortified to 22.5% CP with glutamic acid and essential amino acid [Phe + Tyr, Leu, and Pro from the CGM exceeded NRC (1994) requirements].

[2]The digestible amino acid profile when adequately fortified with Lys, Trp, Thr, Ile, and Val met or exceeded the Illinois ideal amino acid ratios for chicks (Baker and Han, 1994; Baker, 1997) from 0 to 3 wk of age (Lys, 100; Met, 36; Cys, 36; Arg, 105; Val, 77; Thr, 67; Trp, 16; Ile, 67; His, 35; Phe + Tyr, 105; Leu, 109).

[3]Corn gluten meal was analyzed to contain 65.31% CP. It was obtained from ADM corporation, Decatur, IL.

[4]Provided (per kilogram of diet): $Ca_3(PO_4)_2$, 28.0 g; K_2HPO_4, 9.0 g; NaCl, 8.89 g; $MgSO_4 \cdot 7H_2O$, 3.5 g; $ZnCO_3$, 0.10g; $CaCO_3$, 3.0 g; $MnSO_4 \cdot H_2O$, 0.65 g; $FeSO_4 \cdot 7H_2O$, 0.42 g; KI, 40 mg; $CuSO_4 \cdot 5H_2O$, 20 mg; $Na_2MoO_4 \cdot 2H_2O$, 9 mg; H_3BO_3, 9 mg; $CoSO_4 \cdot 7H_2O$, 1 mg; Na_2SeO_3, 0.22 mg.

[5]Provided (per kilogram of diet): thiamin·HCL, 20 mg; niacin, 50 mg; riboflavin, 10 mg; D-Ca-pantothenate, 30 mg; vitamin B_{12}, 0.04 mg; pyridoxine·HCL, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; menadione dimethylpyrimidinol bisulfate, 2 mg; cholecalciferol, 15 μg; retinyl acetate, 1,789 μg; ascorbic acid, 250 mg.

[6]Contributed 27.5 mg/kg bacitracin methylene disalicylate.

The six bioassays were conducted in two series: series 1 involved graded doses of Lys (Assay 1), Trp (Assay 2) and Thr (Assay 3); series 2 (carried out 4 mo after series 1) consisted of graded levels of Lys (Assay 4), Ile (Assay 5) and Val (Assay 6).

Basal Diet

An AA-fortified corn-gluten meal (CGM) diet was formulated to meet or exceed the requirements recommended by NRC (1994) for all nutrients (except the AA under study) for broiler chicks between hatching and 21 d of age (Table 1). The fully fortified CGM diet contained 12% CP from CGM and was supplemented with Lys, Trp, Arg, Thr, Val, Ile, His, Met, and Cys to fulfill the digestible AA profile established by Illinois Ideal Chick Protein ratios for 0 to 21-d-old chicks (Baker and Han, 1994). A digestible Lys level of 1.07% of the diet was used as the reference point for calculating proper levels of the remaining AA to be supplemented. L-glutamic acid was added to bring the CP level of the basal diet to 22.5%. The CGM basal diet was fortified with Lys, Trp, Thr, Ile, and Val to fulfill the digestible AA profile (Baker and Han, 1994) when that AA was not under investigation.

TABLE 2. Essential amino acid composition (as-fed basis) and true digestibility values for corn gluten meal

Amino acid	Composition (%)	Digestibility (%)	True digestible level (%)
Lys	1.11	79.7[1]	0.88
Thr	2.09	90.4[1]	1.89
Trp	0.34	97.0[2]	0.33
Ile	2.49	98.6[1]	2.46
Val	2.87	96.8[1]	2.78

[1]True digestibility was determined in cecetomized roosters (n = 5).

[2]True digestibility value obtained from Rhodiment™ Nutrition Guide (Rhone-Poulenc Animal Nutrition).

Thus, the CGM basal diet was adequate in all AA except for the AA being studied. Corn gluten meal was used because of its limiting AA profile and the large response range for these AA (Peter et al., 2000). Also, the CGM diet, when fortified with adequate levels of crystalline AA, has been shown to be as efficacious as a conventional corn-soybean meal diet for support of chick growth (Peter et al., 2000).

All AA were supplied as L-isomers except Met, which was supplied as the DL-isomer.[3] Free-base forms of AA were used with the exception of Lys (hydrochloride) and His, which was used as L-His•HCl•H_2O. Feed-grade sources were used for Lys•HCl (78.5%), Thr (98.5%), Trp (98%), and Met (99%); the remaining AA were pharmaceutical grade. The true digestibility of free AA was assumed to be 100% (Chung and Baker, 1992b). Levels of Phe + Tyr, Leu, and Pro provided by CGM were allowed to exceed the requirements due to their high levels in CGM. Amino acid additions were made at the expense of glutamic acid and cornstarch. Thus, all diets were formulated to be isonitrogenous within and between assays by varying the quantity of glutamic acid. All vitamins and minerals met or exceeded NRC (1994) requirements.

The CGM used in these assays was analyzed to contain 65.31% CP (AOAC, 1995). Lysine, Thr, Ile, and Val concentrations in CGM (Table 2) were quantified by ion-exchange chromatography using a Beckman 6300 AA auto analyzer[4] following a 24-h acid hydrolysis under a nitrogen atmosphere (Spackman et al., 1958). Tryptophan was quantified similarly, but 24-h LiOH hydrolysis preceded chromatographic analysis for this AA. Performic acid preoxidation preceeded acid hydrolysis in the determination of Met and Cys. True digestibilities of AA in CGM (Table 2) were determined with adult, cecectomized, Single-Comb White Leghorn roosters (Han and Parsons, 1990). Five roosters were selected at random to receive a 30-g crop intubation of CGM, and another five roosters were used as feed-deprived controls to estimate endogenous excretion of AA. Amino acid analysis of excreta was performed by the same procedures as those described for CGM, except that Trp was not quantified in excreta. The true digestibility value for Trp in CGM was obtained from Rodiment™ Nutrition Guide[5]. The total and digestible concentrations of Lys, Trp, Thr, Ile, and Val in CGM are shown in Table 2.

Assay 1 (Lys)

The objective of this assay was to determine the Lys requirement for male broiler chicks from 8 to 21 d of age. The CGM basal diet (Table 1) was made adequate in Trp, Thr, Ile, and Val and was then fortified with increments of L-Lys to achieve digestible Lys levels of 0.677, 0.797, 0.917, 1.038, 1.158, and 1.278%. The total Lys level of the diet containing the highest Lys dose was well above the Lys requirement for 0 to 3-wk-old male chicks (Han and Baker, 1993). Therefore, the Lys levels chosen represented doses covering the linear and plateau regions of the growth curve.

Assay 2 (Trp)

The objective of this assay was to determine the Trp requirement for male broiler chicks from 8 to 21 d of age. The CGM basal diet (Table 1) was made adequate in Lys, Thr, Ile, and Val and was then fortified with increments of L-Trp to achieve true digestible Trp levels of 0.0904, 0.1204, 0.1504, 0.1804, 0.2104, and 0.2404%. These levels of Trp were chosen to represent the linear and plateau regions of the growth curve.

Assay 3 (Thr)

The digestible Thr requirement of 8-to-22-d-old chicks was determined in this assay. The CGM basal diet (Table 1) was made adequate in Lys, Trp, Ile, and Val and was then fortified with increments of L-Thr to achieve true digestible Thr levels of 0.408, 0.488, 0.569, 0.650, 0.731, and 0.812%. The total Thr level of the diet containing the highest Thr dose was well above the NRC (1994) Thr requirement estimate.

Assay 4 (Lys)

This assay was identical to that described for Assay 1, but it was conducted with different chicks at a later time.

[3]Amino acids, except for methionine, were supplied by Biokyowa-NutriQuest, Chesterfield, MO 63017. DL-methionine was supplied by Degussa Corp., Allendale, NJ 07401.

[4]Beckman Instruments Corporation, Palo Alto, CA 94302.

[5]Rhone-Poulenc Animal Nutrition, Antony Cedex, France 92164.

Assay 5 (Ile)

This assay was done to determine the digestible Ile requirement of male broiler chicks from 8 to 21 d of age. The CGM basal diet (Table 1) was made adequate in Lys, Trp, Thr, and Val and was then fortified with increments of L-Ile to achieve true digestible Ile levels of 0.450, 0.550, 0.650, 0.750, 0.850 and 0.950%. These levels were chosen to represent the linear and plateau regions of the growth curve.

Assay 6 (Val)

The objective of this assay was to determine the digestible Val requirement of 8-to-21-d-old male broiler chicks. The CGM basal diet (Table 1) was supplemented with Lys, Trp, Thr, and Ile and was then fortified with incremental levels of L-Val to arrive at true digestible Val levels of 0.510, 0.620, 0.730, 0.840, 0.950 and 1.060%. The total Val level in the diet containing the highest dose of Val was well above the NRC (1994) Val requirement estimate.

Statistical Analysis

Data for weight gain and gain:feed were subjected to ANOVA procedures appropriate for completely randomized designs (Steel and Torrie, 1980) by using the General Linear Model (GLM) procedure of SAS® software (SAS Institute, 1990). Digestible AA requirements were estimated by broken-line methodology (Robbins et al., 1979) and by fitting the data to a quadratic response curve (Draper and Smith, 1981) and then determining the dietary AA level required for maximal weight gain and feed efficiency (X at maximum Y value of quadratic response curve). A value of 90% of the X value required for maximal Y was selected as a subjective estimate of the requirement. An objective estimate of digestible AA requirements from the quadratic models was determined by establishing the first point at which the quadratic response curve intersected the plateau value established from the one-slope fitted broken-line. The intercept value of X can be calculated using the quadratic regression equation once the plateau value (Y) of the broken-line is determined. Because fits of the broken-line responses were excellent, and because the breakpoints determined from the broken-line fits represented minimal but objective requirement estimates, these values were deemed most appropriate for calculation of ideal ratios. Thus, ratios were based on the higher of broken-line requirement estimates for gain and gain:feed.

RESULTS

Assay 1 (Lys)

Weight gain and feed efficiency responded quadratically ($P < 0.01$) to graded levels of digestible Lys (Table 3). Broken-line least-squares analysis (single-slope model) predicted a break-point in weight gain and feed efficiency at 0.846 and 0.964% digestible Lys, respectively. The broken-line plot of feed efficiency is shown in Figure 1 (r^2 = 0.885). A quadratic equation also described the weight gain and feed efficiency responses well. The equation for feed efficiency (Figure 1) was Y [gain(g):feed(kg)] = -300.2 + 1,746.8X - 760.4X^2 (r^2 = 0.899) with X being the true digestible Lys level (% of diet). The X value required to achieve maximal Y was obtained by dividing the derivative of Y by the derivative of X. The level of digestible Lys that maximized weight gain and feed efficiency (upper asymptote) was calculated to be 1.08 and 1.15% of the diet, with 90% of these values being 0.97 and 1.03%, respectively. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line for weight

TABLE 3. Growth performance of young chicks fed graded levels of digestible lysine (Assay 1)[1]

True digestible Lys[2] (% of diet)	Lys intake (g)	Weight gain[3] (g)	Gain:feed[3] (g/kg)
0.677	2.44	193	534
0.797	3.57	273	610
0.917	4.27	309	665
1.038	4.69	309	684
1.158	5.01	308	712
1.278	5.56	299	687
Pooled SEM		13	11

[1]Data are means of four pens of four male Columbian × New Hampshire chicks from 8 to 21 d posthatching; average initial weight was 96 g.

[2]Lysine was provided as feed-grade L-Lys•HCl. The Lys-deficient basal diet (Table 1) was fortified with Trp, Thr, Ile, and Val to provide digestible levels of these amino acids at 0.19, 0.74, 0.78, and 0.92%, respectively.

[3]Quadratic and broken-line response ($P < 0.01$).

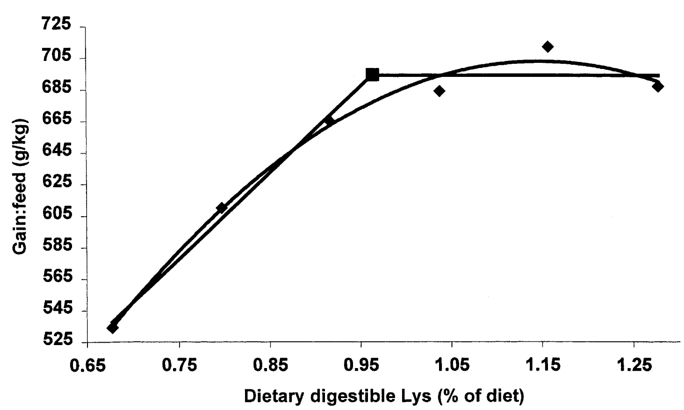

FIGURE 1. Fitted broken-line and quadratic plot of gain:feed as a function of true digestible Lys in the diet (Assay 1). The minimal true digestible Lys requirement determined by broken-line analysis using least squares methodology was 0.964% [Y = 694.4 + 549.0 (X < 0.964); r^2 = 0.885]. The pen means data from Table 3 also were fitted to a quadratic regression equation Y= -300.2 + 1,746.8X – 760.4X^2 (r^2 = 0.899). The level of true digestible Lys that maximized feed efficiency (i.e., upper asymptote) was calculated to be 1.149% of the diet, with 90% of this value being 1.034%. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line occurred at 1.040% true digestible Lys.

TABLE 4. Growth performance of young chicks fed graded levels of digestible tryptophan (Assay 2)[1]

True digestible Trp[2] (% of diet)	Trp intake (g)	Weight gain[3] (g)	Gain:feed[3] (g/kg)
0.0904	0.201	73	327
0.1204	0.377	168	535
0.1504	0.644	277	646
0.1804	0.782	291	671
0.2104	0.884	307	730
0.2404	1.084	311	689
Pooled SEM		13	34

[1]Data are means of four pens of four male Columbian × New Hampshire chicks from 8 to 21 d posthatching; average initial weight was 96 g.

[2]Tryptophan was provided as feed-grade L-Trp. The Trp-deficient basal diet (Table 1) was fortified with Lys, Thr, Ile, and Val to provide digestible levels of these amino acids at 1.18 0.74, 0.78, and 0.92%, respectively.

[3]Quadratic and broken-line response ($P < 0.01$).

TABLE 5. Growth performance of young chicks fed graded levels of digestible threonine (Assay 3)[1]

True digestible Thr[2] (% of diet)	Thr intake (g)	Weight gain[3] (g)	Gain:feed[3] (g/kg)
0.408	1.26	191	519
0.488	2.23	280	612
0.569	2.82	327	659
0.650	3.30	346	680
0.731	3.60	322	653
0.812	3.92	322	665
Pooled SEM		10	16

[1]Data are means of four pens of four male Columbian × New Hampshire chicks from 8 to 22 d posthatching; average initial weight was 103 g.

[2]Threonine was provided as feed-grade L-Thr. The Thr-deficient basal diet (Table 1) was fortified with Lys, Trp, Ile, and Val to provide digestible levels of these amino acids at 1.18, 0.19, 0.78 and 0.92%, respectively.

[3]Quadratic and broken-line response ($P < 0.01$).

gain and gain:feed occurred at 0.942 and 1.040% true digestible Lys, respectively (Figure 1).

Assay 2 (Trp)

Weight gain and feed efficiency responded quadratically ($P < 0.01$) to graded levels of digestible Trp (Table 4). Broken-line least-squares analysis predicted a breakpoint in weight gain at 0.159% and feed efficiency at 0.157% digestible Trp (Figure 2). The quadratic equation for weight gain (Figure 2) was $Y = -423.7 + 6{,}971.4X - 16{,}403.8X^2$ ($r^2 = 0.922$) with X being the true digestible Trp level (% of diet). The X values required to achieve maximal Y from the quadratic equations for weight gain and gain:feed, respectively, were calculated to be 0.21 and 0.20% of the diet, with 90% of these values being 0.19 and 0.18%. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line for weight gain and gain:feed occurred at 0.183 and 0.176% true digestible Trp, respectively.

Assay 3 (Thr)

Weight gain and feed efficiency responded quadratically ($P < 0.01$) to graded levels of digestible Thr (Table 5). Broken-line least-squares analysis predicted breakpoints in weight gain and feed efficiency at 0.535 and 0.533% digestible Thr, respectively. The broken-line plot of weight gain is shown in Figure 3 ($r^2 = 0.867$). The quadratic equation for weight gain (Figure 3) was $Y = -559.3 + 2{,}658.6X - 1{,}953.5X^2$ ($r^2 = 0.855$) with X being true digestible Thr level (% of diet). The X values required to achieve maximal Y for gain and feed efficiency, respec-

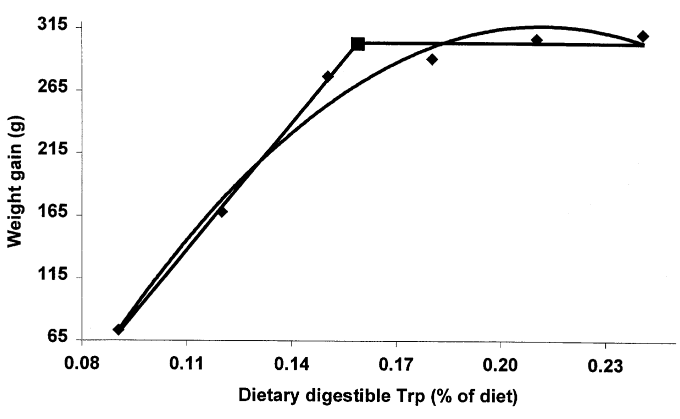

FIGURE 2. Fitted broken-line and quadratic plot of 13-d weight gain as a function of true digestible Trp in the diet (Assay 2). The minimal true digestible Trp requirement determined by broken-line analysis using least squares methodology was 0.159% [$Y = 302.8 + 3{,}395.8$ ($X < 0.159$); $r^2 = 0.934$]. The pen means data from Table 4 also were fitted to a quadratic regression equation $Y = -423.7 + 6{,}971.4X - 16{,}403.8X^2$ ($r^2 = 0.922$). The level of true digestible Trp that maximized weight gain (i.e., upper asymptote) was calculated to be 0.212% of the diet, with 90% of this value being 0.191%. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line occurred at 0.183% true digestible Trp.

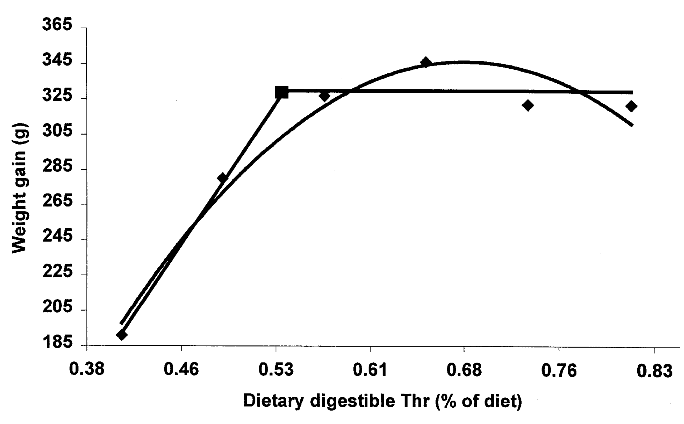

FIGURE 3. Fitted broken-line and quadratic plot of 14-d weight gain as a function of true digestible Thr in the diet (Assay 3). The minimal true digestible Thr requirement determined by broken-line analysis using least squares methodology was 0.535% [$Y = 328.8 + 1{,}084.4$ ($X < 0.535$), $r^2 = 0.867$]. The pen means data from Table 5 also were fitted to a quadratic regression equation: $Y = -559.3 + 2{,}658.6X - 1{,}953.5X^2$ ($r^2 = 0.855$). The level of true digestible Thr that maximized weight gain (i.e., upper asymptote) was calculated to be 0.680% of the diet, with 90% of this value being 0.612%. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line occurred at 0.590% true digestible Thr.

TABLE 6. Growth performance of young chicks fed graded levels of digestible lysine (Assay 4)[1]

True digestible Lys[2] (% of diet)	Lys intake (g)	Weight gain[3] (g)	Gain:feed[3] (g/kg)
0.677	2.48	192	525
0.797	3.87	288	593
0.917	4.70	324	632
1.038	5.36	334	647
1.158	5.60	325	672
1.278	6.39	332	664
Pooled SEM		9	12

[1]Data are means of four pens of four male Columbian × New Hampshire chicks from 8 to 21 d posthatching; average initial weight was 90 g.

[2]Lysine was provided as feed-grade L-Lys·HCl. The Lys-deficient basal diet (Table 1) was fortified with Trp, Thr, Ile, and Val to provide digestible levels of these amino acids at 0.19, 0.74, 0.78, and 0.92%, respectively.

[3]Quadratic and broken-line response ($P < 0.01$).

TABLE 7. Growth performance of young chicks fed graded levels of digestible isoleucine (Assay 5)[1]

True digestible Ile[2] (% of diet)	Ile intake (g)	Weight gain[3] (g)	Gain:feed[3] (g/kg)
0.450	1.47	192	587
0.550	2.14	265	680
0.650	2.67	295	717
0.750	3.14	291	694
0.850	3.48	291	711
0.950	3.88	291	712
Pooled SEM		8	10

[1]Data are means of four pens of four male Columbian × New Hampshire chicks from 8 to 21 d posthatching; average initial weight was 100 g.

[2]Isoleucine was provided as analytical grade L-Ile. The Ile-deficient basal diet (Table 1) was fortified with Lys, Trp, Thr, and Val to provide digestible levels of these amino acids at 1.18, 0.19, 0.74, and 0.92%, respectively.

[3]Quadratic and broken-line response ($P < 0.01$).

tively, were 0.68 and 0.69% of the diet, with 90% of these values being 0.61 and 0.62%, respectively. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line for weight gain and gain:feed occurred at 0.590 and 0.598% true digestible Thr, respectively.

Assay 4 (Lys)

The Lys responses in this assay (Table 6; Figure 4) were similar to those observed in Assay 1, which also involved graded levels of digestible Lys. Broken-line least squares analysis predicted break-points in weight gain and feed efficiency at 0.849 and 0.956% digestible Lys, respectively. From the quadratic regression equations, the X value required to achieve maximal Y was 1.10% for weight gain and 1.12% for feed efficiency, with 90% of these upper asymptotic values being 0.99 and 1.01%, respectively. The first intercept X value of the broken line (on the plateau) and the quadratic fitted line for weight gain and gain:feed occurred at 0.962 and 1.01% digestible Lys, respectively (Figure 4). These requirement estimates, and those determined from the intercept of the broken lines, were in close agreement with those determined in Assay 1.

Assay 5 (Ile)

Weight gain and feed efficiency responded quadratically ($P < 0.01$) to graded levels of digestible Ile (Table 7). Broken-line least-squares analysis predicted breakpoints in weight gain at 0.589% and feed efficiency at 0.581% digestible Ile. The broken-line plot of weight gain ($r^2 = 0.876$) is shown in Figure 5. The quadratic equation for weight gain (Figure 5) was Y = -239.1 + 1,365.4X -

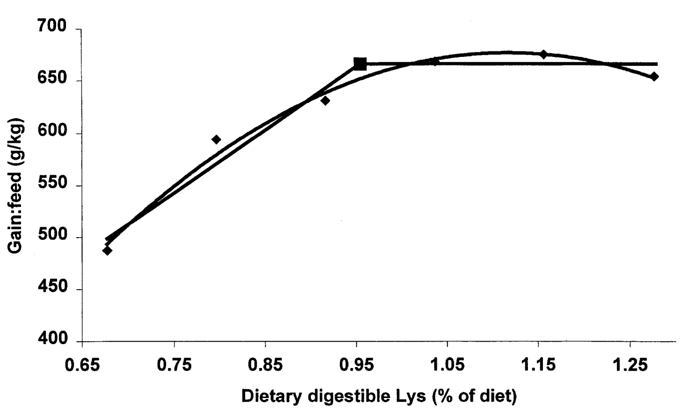

FIGURE 4. Fitted broken-line and quadratic plot of gain:feed as a function of true digestible Lys in the diet (Assay 4). The minimal true digestible Lys requirement determined by broken-line analysis using least squares methodology was 0.956% [$Y = 665.83 + 598.96(X < 0.956)$; $r^2 = 0.870$]. The pen means data from Table 6 also were fitted to a quadratic regression equation ($Y = -502.70 + 2{,}108.79X - 942.32X^2$; $r^2 = 0.897$). The level of true digestible Lys that maximized gain:feed (i.e., upper asymptote) was calculated to be 1.12% of the diet, with 90% of this value being 1.01%. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line occurred at 1.01% true digestible Lys.

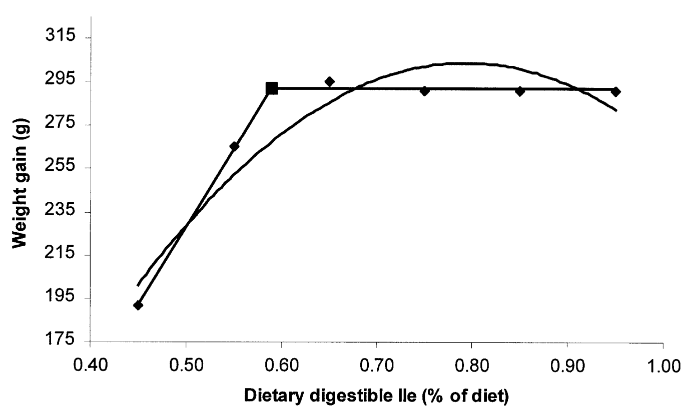

FIGURE 5. Fitted broken-line and quadratic plot of 13-d weight gain as a function of true digestible Ile in the diet (Assay 5). The minimal true digestible Ile requirement determined by broken-line analysis using least squares methodology was 0.589% [$Y = 292.0 + 722.5$ ($X < 0.589$); $r^2 = 0.876$]. The pen means data from Table 7 also were fitted to a quadratic regression equation ($Y = -239.1 + 1{,}365.4X - 858.9X^2$; $r^2 = 0.810$). The level of true digestible Ile that maximized weight gain (i.e., upper asymptote) was calculated to be 0.795% of the diet, with 90 % of this value being 0.715%. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line occurred at 0.679% true digestible Ile.

$858.9X^2$ ($r^2 = 0.810$), with X being true digestible Ile level (% of the diet). The X values required to achieve maximal Y from the quadratic equations for weight gain and gain:feed, respectively, were calculated to be 0.80 and 0.80% digestible Ile, with 90% of these values being 0.72 and 0.72%. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line for weight gain and gain:feed occurred at 0.679 and 0.685% true digestible Ile, respectively.

Assay 6 (Val)

Weight gain and feed efficiency responded quadratically ($P < 0.01$) to graded levels of digestible Val (Table 8). Broken-line least-squares analysis predicted breakpoints in weight gain and feed efficiency at 0.744 and 0.743% digestible Val, respectively. The broken-line plot of weight gain ($r^2 = 0.963$) is shown in Figure 6. The quadratic equation for weight gain (Figure 6) was Y = 618.42 + 2,003.92X - $1{,}088.40X^2$ ($r^2 = 0.950$), with X being true digestible Val level (% of the diet). The X values required to achieve maximal Y for gain and feed efficiency, respectively, were 0.92 and 0.93% of the diet, with 90% of these upper asymptotic values occurring at 0.83 and 0.84%, respectively. The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line for weight gain and gain:feed occurred at 0.811 and 0.816% true digestible Val, respectively.

TABLE 8. Growth performance of young chicks fed graded levels of digestible valine (Assay 6)[1]

True digestible Val[2] (% of diet)	Val intake (g)	Weight gain[3] (g)	Gain:feed[3] (g/kg)
0.510	1.28	119	476
0.620	2.09	201	597
0.730	2.96	280	690
0.840	3.45	288	702
0.950	3.99	297	707
1.060	4.28	288	713
Pooled SEM		7	16

[1]Data are means of four pens of four male Columbian × New Hampshire chicks from 8 to 21 d posthatching; average initial weight was 100 g.

[2]Valine was provided as analytical grade L-Val. The Val-deficient basal diet (Table 1) was fortified with Lys, Trp, Thr, and Ile to provide digestible levels of these amino acids at 1.18, 0.19, 0.74, and 0.78%, respectively.

[3]Quadratic and broken-line response ($P < 0.01$).

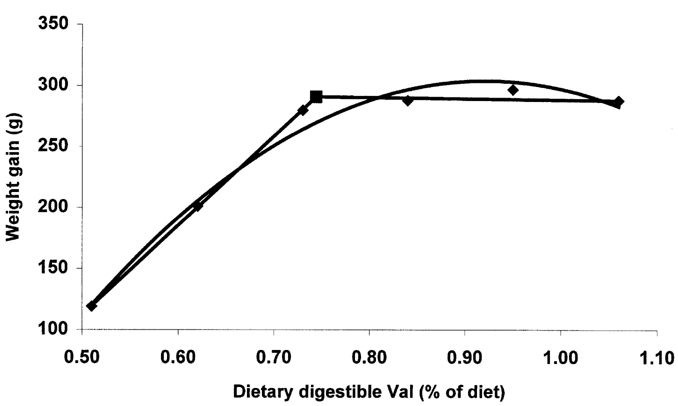

FIGURE 6. Fitted broken-line and quadratic plot of 13-d weight gain as a function of true digestible Val in the diet (Assay 6). The minimal true digestible Val requirement determined by broken-line analysis using least squares methodology was 0.744% [Y = 290.83 + 731.82 (X < 0.744); $r^2 = 0.963$]. The pen means data from Table 8 also were fitted to a quadratic regression equation (Y = 618.42 + 2,003.92X – $1{,}088.40X^2$; $r^2 = 0.950$.). The level of true digestible Val that maximized weight gain (i.e., upper asymptote) was calculated to be 0.921% of the diet, with 90 % of this value being 0.829%, The first intercept X value of the broken-line (on the plateau) and the quadratic fitted line occurred at 0.811% true digestible Val.

TABLE 9. Summary of true digestible requirement estimates (% of diet) for male chicks fed a fortified corn gluten meal semipurified diet during the second and third week posthatch: extrapolation to ideal ratios relative to lysine[1]

Amino acid	Weight gain	Gain:feed	Ratio[2]
Lysine	0.848	0.960	100
Tryptophan	0.159	0.157	16.6
Threonine	0.535	0.533	55.7
Isoleucine	0.589	0.581	61.4
Valine	0.744	0.743	77.5

[1]Based on broken-line requirement estimates from data in Tables 3, 4, 5, 6, 7, and 8. The Lys requirements represent average values from Assay 1 (Table 3) and Assay 4 (Table 6).

[2]The requirement used for ratio calculation was taken to be the higher of individual estimates for gain and gain:feed ratio.

DISCUSSION

Amino acid requirements established using experimental diets containing minimal intact protein and a plethora of nitrogen from indispensable and dispensable AA often do not result in requirement estimates that can be extrapolated to practice. Our AA assay diets contained about one half of the CP as intact protein (CGM) and the other half as free AA. Thus, even though our assay diets contained CP and energy levels similar to conventional corn-soybean meal diets, and even though our requirement estimates were based on true digestibility values, we still believe that assay diets such as ours herein often yield (for unexplained reasons) requirement estimates that cannot be directly applied to the conventional corn-soybean meal diets that are used in practice. Hence, we wish to make it clear that our primary objective was to establish accurate ideal AA ratios rather than to define requirements per se for the five AA studied herein.

The Lys requirement determined for optimum gain:feed was substantially higher than that determined for maximal weight gain, regardless of which curve-fitting procedure was used to predict the requirement (Table 9). Han and Baker (1993; 1994) and Mack et al. (1999) came to the same conclusion. The higher Lys requirement for gain:feed than for gain is in stark contrast to the broken-line requirement estimates for Trp, Thr, Ile, and Val established herein (Table 9) and by Mack et al. (1999). Thus, Trp, Thr, Ile and Val requirements for optimal gain were almost identical to the predicted requirements for optimal feed efficiency. With Lys, when gain and voluntary feed intake reach their highest point, gain remains essentially constant but feed intake decreases as addi-

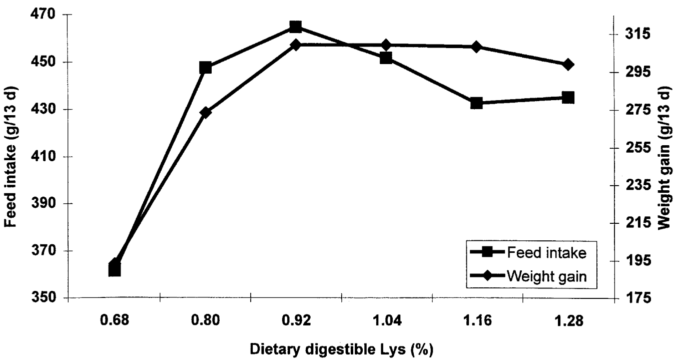

FIGURE 7. Plot of feed intake and weight gain of chicks fed graded levels of digestible Lys in Assay 1.

tional Lys is incremented (Figure 7). Why this occurs with Lys but not with most other AA is not known. Previous work with SAA suggests that the requirement for SAA may also be higher for maximal feed efficiency than for maximal weight gain (Schutte and Pack, 1995; Baker et al., 1996; Mack et al., 1999)

Our ideal Trp:Lys ratio estimate of 16.6% is very similar to our original estimate of 16% for 0 to 3-wk-old chicks (Baker and Han, 1994; Baker, 1997). Mack et al. (1999) concluded that the ideal Trp:Lys ratio is 19%, but this ratio was based on the digestible Trp requirement for weight gain divided by the digestible Lys requirement for weight gain rather than feed efficiency. If one uses their broken-line Lys requirement estimate for feed efficiency as a base, which we feel is most defendable, Mack et al. (1999) would have arrived at an ideal Trp:Lys ratio of 17% instead of 19%. However, because they did not obtain significant ($P < 0.05$) gain or feed efficiency responses to Trp, their data did not lend themselves to an accurate estimate of the ideal Trp:Lys ratio.

Using the 16.6% ideal Trp:Lys ratio determined herein together with a best estimate of the digestible Lys requirement (1.07%) for broiler chicks during the first 21 d posthatching (mixed sex feeding) as determined previously (Han and Baker, 1991; 1993), one arrives at a digestible Trp requirement estimate of 0.178% of a diet containing 3,200 kcal ME/kg. This estimate agrees well with the requirement estimate of 0.183% obtained by determining the first intercept X value of the broken line (on the plateau) and the quadratic fitted line (Figure 3). With an estimated Trp digestibility of 86% in typical corn-soybean broiler diets (Rhone-Poulenc, 1993),[5] the total Trp requirement is calculated to be 0.21% (0.178/0.86) for chicks consuming corn-soybean meal diets. This value is in good agreement with the NRC (1994) Trp requirement estimate of 0.20% for chicks during the first 3 wk posthatch.

The results obtained with Thr were somewhat surprising in that the predicted ideal Thr:Lys ratio was only 55.7% (Table 9), far lower than our original estimate of 67% (Baker and Han, 1994; Baker, 1997). Mack et al. (1999) predicted an ideal Thr:Lys ratio of 63% using broken-line gain requirements for Thr and Lys. Using their gain:feed requirement for Lys and their gain requirement for Thr, however, one can calculate that their predicted ideal Thr:Lys ratio would have been only 59%. These lower-than-expected ratios for Thr:Lys may indicate that the NRC (1994) Thr requirement (0.80% total, and a calculated value of 0.70% digestible Thr) for 0 to 3-wk-old chicks is too high. By using both (total) requirement estimates and true digestibility values of NRC (1994), but using a more accepted estimate of the Lys requirement of 1.20% total and 1.07% digestible Lys for mixed sex feeding (Han and Baker, 1991; 1993), the ideal digestible Thr:Lys ratio is predicted by NRC (1994) to be 65%. Based on the fact that empirical evidence is strong in support of the Lys requirement but weaker in support of the Thr requirement of 0 to 3-wk-old chicks, and also based on the ratio estimates herein and those of Mack et al. (1999), it appears that the ideal Thr:Lys ratio does not exceed 60%, and therefore the predicted Thr requirement may not exceed 0.72% (0.64% digestible) Thr. One could interpret the Thr work of Smith and Waldroup (1988) and Kidd et al. (1997) as being in support of this lower Thr requirement for broiler chicks during the first 3 wk posthatch.

Using the higher of weight gain and gain:feed broken-line requirement estimates for Ile (0.589%) and Lys (0.960%) yields an Ile:Lys ideal ratio estimate of 61.4% (Table 9). This value is lower than our original estimate of 67% (Baker, 1997). Extrapolation of the ideal Ile:Lys ratio of 61.4% to a predicted Ile requirement for chicks fed a corn-soybean meal diet during the first 3 wk posthatching suggests that the NRC (1994) Ile requirement estimate (0.80% total, 0.73% digestible Ile) is too high. Thus, if one assumes that the digestible Lys requirement of 0 to 3-wk-old chicks (mixed sex) is 1.07% (Han and Baker, 1993), the predicted digestible Ile requirement would be 0.66%, a value lower than the 0.73% digestible Ile requirement estimate of NRC (1994).

The estimated ideal Val:Lys ratio was 77.5% based on the broken-line requirement estimates of 0.744% for Val (weight gain) and 0.960% for Lys (feed efficiency). This ideal ratio estimate for Val is in close agreement with our earlier estimate of 77% (Baker, 1997) and with that estimated from the data of Mack et al. (1999), i.e., 76%. The relatively high ideal ratio of Val:Lys perhaps explains why Val is among the four most limiting AA in reduced protein corn-soybean meal diets for broiler chicks (Fernandez et al., 1994).

We considered the breakpoint of fitted broken-lines the best basis for predicting ideal ratios, and Mack et al. (1999) came to the same conclusion. The fits (r^2) of our broken lines were excellent. Moreover, breakpoints of fitted broken lines predict minimal requirement values, and this is viewed as desirable for calculating AA ratios. Indeed, ratios should be based not on the Lys level being fed, which perhaps allows for a safety factor and is therefore in excess, but on a minimally required level of Lys such as that established by the breakpoint of fitted broken lines. Another advantage of the fitted broken-line approach is that the inflection point of the fitted line is established objectively rather than subjectively. The disadvantage of quadratic fits for AA requirement studies is that quadratic fits do not establish objective breakpoints.

We are introducing herein a new objective means of predicting requirements, using the plateau value from one-slope fitted broken lines and the quadratic fits to the same data. For most data sets that cover the linear and plateau regions of the growth curve, best-fit quadratic curves intersect broken lines in at least three places: once (or twice) on the ascending portion and twice on the plateau portion (Figures 1 to 6). The objective Y plateau value (gain or gain:feed) from best-fit broken lines can be substituted into the best-fit quadratic equation so that the equation $0 = aX^2 + bX + c$ is obtained. Then, one can calculate the first and the second intercept (X) values for where the quadratic curve intersects the plateau of the broken line. Software programs are available to easily obtain broken-line and quadratic equations using SAS® software (SAS Institute, 1990) and also to calculate both intercept X values on the plateau of the broken-line response curve. The first intercept value represents, in our judgement, an excellent representation of the requirement. Moreover, that this intercept value is obtained objectively is a distinct advantage. In most cases, as was the case herein, the first intercept X value of the broken-line plateau and the quadratic fitted curve predicts a requirement value close to that predicted by taking 90% of the upper asymptote value of the quadratic equation.

Using the first intercept X value on the plateau of broken lines with the fitted quadratic curves yielded an average digestible requirement estimate (Assays 1 and 4) of 1.025% for Lys (gain:feed). Similar estimates using this methodology were 0.183 (Trp), 0.590 (Thr), 0.679 (Ile), and 0.811 (Val) using weight gain as the response criterion. These requirement estimates ratioed to the Lys requirement calculate to be 17.9% for Trp, 57.6% for Thr, 66.2% for Ile, and 79.1% for Val, estimates that are slightly higher than those predicted by using breakpoints of fitted broken lines. We believe, however, that the breakpoints of fitted broken lines are the most defendable means of determining AA ratios.

The data herein illustrate the complexities of establishing accurate AA ratios. Decisions such as what output criterion to use (i.e., only gain, only gain:feed, or whichever of these yields the most defendable requirement), and what statistical means is best used to establish the requirement can either increase or decrease the predicted ideal ratios. Clearly, using the gain requirement instead of the gain:feed requirement for Lys will increase the calculated ratios substantially. For example, using the broken-line gain requirement for Lys in our study increases the Trp:Lys ratio from 16.6% to 18.8%, the Thr:Lys ratio from 55.7% to 63.1%, the Ile:Lys ratio from 61.5 to 69.5%, and the Val:Lys ratio from 77.5 to 87.7% (Table 9). Because the feed efficiency requirement for Lys is most defendable, we feel that the ratio predictions of 16.6% for Trp:Lys, 55.7% for Thr:Lys, 61.4% for Ile:Lys, and 77.5% for Val:Lys are best estimates of ideal ratios that can be applied to practice. Whether these ratios established in chicks during the 2nd and 3rd week posthatch can be applied to older broiler chicks is problematic. Edwards et al. (1999), however, found that the maintenance requirement for Lys was surprisingly high, and Emmert and Baker (1997) suggested that the Trp:Lys and SAA:Lys ratios may not increase as chicks progress from hatching to 8-wk posthatching. They also postulated that the Thr:Lys ratio may increase only slightly as broiler chicks increase in age and weight.

REFERENCES

Association of Official Analytical Chemists. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington, DC.

Baker, D. H. 1997. Ideal amino acid profiles for swine and poultry and their applications in feed formulation. BioKyowa Technol. Rev. 9:1–24.

Baker, D. H., S. R. Fernandez, D. M. Webel, and C. M. Parsons. 1996. Sulfur amino acid requirement and cystine replacement value of broiler chicks during the period three to six weeks posthatching. Poult. Sci. 75:737–742.

Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks post-hatching. Poult. Sci. 73:1441–1447.

Chung, T. K., and D. H. Baker. 1992a. Ideal amino acid pattern for 10-kilogram pigs. J. Anim. Sci. 70:3102–3111.

Chung, T. K., and D. H. Baker. 1992b. Apparent and true digestibility of a crystalline amino acid mixture and of casein comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. J. Anim. Sci. 70:3781–3790.

Draper, N. R., and H. Smith. 1981. Applied Regression Analysis. 2nd ed. John Wiley and Sons, New York.

Edwards, H. M., III, S. R. Fernandez, and D. H. Baker. 1999. Maintenance lysine requirement and efficiency of using lysine for accretion of whole-body lysine and protein in young chicks. Poult. Sci. 78:1412–1417.

Emmert, J. L., and D. H. Baker. 1997. Use of the ideal protein concept for precision formulation of amino acid levels in broiler diets. J. Appl. Poult. Res. 6:462–470.

Fernandez, S. R., S. Aoyagi, Y. Han, C. M. Parsons, and D. H. Baker. 1994. Limiting order of amino acids in corn and soybean meal for growth of the chick. Poult. Sci. 73:1887–1896.

Han, Y., and D. H. Baker. 1991. Lysine requirement of fast and slow growing broiler chicks. Poult. Sci. 70:2108–2114.

Han, Y., and D. H. Baker. 1993. Effects of sex, heat stress, body weight and genetic strain on the lysine requirement of broiler chicks. Poult. Sci. 72:701–708.

Han, Y., and D. H. Baker. 1994. Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. Poult. Sci. 73:1739–1745.

Han, Y., and C. M. Parsons. 1990. Determination of available amino acid and energy in alfalfa meal, feather meal and poultry by-products by various methods. Poult. Sci. 69:1544–1552.

Kidd, M. T., B. J. Kerr, and N. B. Anthony. 1997. Dietary interactions between lysine and threonine in broilers. Poult. Sci. 76:608–614.

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.

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

Peter, C. M., Y. Han, S. D. Boling-Frankenbach, C. M. Parsons, and D. H. Baker. 2000. Limiting order of amino acids and the effects of phytase on protein quality in corn gluten meal fed to young chicks. J. Anim. Sci. 78:2150–2156.

Robbins, K. R., H. W. Norton, and D. H. Baker. 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109:1710–1714.
SAS. 1990. User's Guide. Release 6.08. SAS Institute Inc., Cary, NC.
Schutte, J. B., and M. Pack. 1995. Sulfur amino acid requirement of broiler chicks from fourteen to thirty-eight days of age. 1. Performance and carcass yield. Poult. Sci. 74:480–487.
Smith, N. K., and P. Waldroup. 1988. Investigations of threonine requirements of broiler chicks fed diets based on grain sorghum and soybean meal. Poult. Sci. 67:108–112.
Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30:1190–1206.
Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., New York.
Wang, T. C., and M. F. Fuller. 1989. The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. Br. J. Nutr. 62:77–89.