Response of pullets to digestible lysine intake

J.A. de Araujo[1], N.K. Sakomura[1], E.P. da Silva[1], J.C. De Paula Dorigam[1], D.C. Zanardo Donato[1], J.H.V. da Silva[2], J.B.K. Fernandes[3]

[1]Department of Animal Science, Faculty of Agrarian and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil
[2]Department of Agriculture, Centre for Human, Social, and Agrarian Sciences, Bananeiras, Paraíba, Brazil
[3]Aquaculture Centre, Faculty of Agrarian and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil

ABSTRACT: The objective was to determine the efficiency of utilization of lysine and to describe the responses of pullets to different digestible lysine intakes using three mathematical functions to estimate an optimal intake maximizing body weight gain and feed conversion ratio. The trials were conducted using 2–6-, 8–12-, and 14–18-week-old birds and a completely randomized experimental design with eight treatments and six replicates. The digestible lysine levels ranged 3.20–10.67 g/kg (in 2–6-week-old animals), 2.24–7.48 g/kg (in 8–12-week-old animals), and 1.73–5.78 g/kg (in 14–18-week-old animals) and were obtained using a dilution technique. The efficiency of utilization was determined by a linear regression between lysine deposition and intake for pullets aged 2–4, 8–10, and 14–16 weeks. Three mathematical functions (quadratic polynomial, broken-line, and broken-line with quadratic ascending function) were used to fit the body weight gain and feed conversion responses. The optimal digestible lysine intake was obtained from the first intercept of the quadratic curve with the broken-line plateau. Based on the body weight gain, the responses obtained from the broken-line function and the broken-line with quadratic ascending were similar to those from the quadratic polynomial function. The feed conversion ratio from the first intercept of the quadratic curve with the broken-line plateau was similar to the value obtained from the broken-line with quadratic ascending function only in 2–6-week-old animals. The digestible lysine intakes required to optimize the body weight gain and feed conversion ratio responses were 202, 338, and 300 and 146, 312, and 259 mg/day and the efficiencies were 80, 76, and 80% for 2–6-, 8–12-, and 14–18-week-old animals, respectively.

Keywords: age; amino acid; feed conversion ratio; growth; mathematical functions

INTRODUCTION

The nutritional levels set in the growth phase should promote maximal growth potential and adequate body condition at the first egg laying (Emmans and Fisher, 1986; Martin et al., 1994; Leeson and Summers, 2005). The amino acid lysine (Lys) is typically used as a reference amino acid to establish the overall amino acid requirements, as suggested by the ideal protein concept (Baker et al., 2002). Due to growth pattern differences in laying hen strains (Martin et al., 1994), efficient nutritional programs should be developed considering the levels of specific amino acids for each genotype.

Defining digestible Lys requirements for pullets in the growth phase has traditionally been based on the dose response method (Silva et al., 2000a, b; Halle, 2002). The responses obtained by this methodology are extrapolated to the population using mathematical functions (Pesti et al., 2009).

The theoretical assumptions of each model can aid in the empirical interpretation of the responses to different nutrient intakes that directly affect the optimal intake (Pesti et al., 2009).

Updating the digestible Lys requirements would redefine the target dietary protein amino acid profile. The primary reasons for precise establishing the amino acid requirements include cost, as amino acids are the most expensive nutrients per unit, and the environmental pressures on nitrogen losses and caloric stress. When birds were fed low quality protein diets, heat production increased because di-

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etary amino acids were poorly incorporated into body proteins (Coon, 2004). Therefore, this study aimed to determine the efficiency of utilization of lysine and to describe the responses of pullets to different digestible lysine intakes using three mathematical functions to estimate an optimal intake maximizing body weight gain and feed conversion ratio.

MATERIAL AND METHODS

The experiments were conducted at the Laboratory of Poultry Sciences of the Department of Animal Science, Faculty of Agrarian and Veterinary Sciences, UNESP, Jaboticabal, São Paulo, Brazil. The experimental procedures used in this study were approved by the Committee of Eethics and Animal Welfare (protocol No. 007125-08) of the same institution. The trials were performed in three phases (in 2–6-, 8–12-, and 14–18-week-old animals). A total of 1488 Dekalb white pullets were used for the entire period. The birds used during the breeding (8–12-week-old) and rearing (14–18-week-old) periods were raised simultaneously in a shed with controlled temperature and ventilation until weeks 7 and 13, respectively, and were fed diets formulated to meet their requirements as recommended by Rostagno et al. (2005). At the beginning of the experiments, the birds were chosen based on body weight.

Table 1. Composition of the experimental diets (%)

Ingredients	P1		P2		P3	
	L7	N-free	L7	N-free	L7	N-free
Corn	59.053	–	65.784	–	72.814	–
Soybean meal	36.352	–	22.206	–	10.160	–
Wheat bran	–	–	8.639	–	10.089	–
Corn gluten meal (60%)	–	–	–	–	3.689	–
Dicalcium phosphate	1.753	2.333	1.464	2.008	1.052	1.645
Limestone	1.057	0.655	10.111	0.518	1.251	0.717
Soybean oil	0.728	6.000		6.000	0.180	6.000
Sodium chloride	0.405	0.417	0.354	0.366	0.329	0.340
DL-Methionine (99%)	0.303	–	0.198	–	0.072	–
L-Lysine (78%)	–	–	–	–	0.017	–
Vitamin supplement[1]	0.100	0.100	0.100	0.100	0.100	0.100
L-Threonine (99%)	0.095	–	–	–	–	–
Choline chloride (70%)	0.070	0.070	0.070	0.070	0.070	0.070
Mineral supplement[2]	0.050	0.050	0.050	0.050	0.050	0.050
L-Valine (99%)	0.024	–	–	–	–	–
L-Tryptophan (99%)	–	–	–	–	0.019	–
L-Isoleucine (99%)	–	–	0.022	–	0.008	–
Antioxidant	0.010	0.010	0.010	0.010	0.010	0.010
Washed sand	–	12.694	–	12.627	–	12.897
Corn starch	–	49.671	–	49.671	–	49.671
Sugar	–	15.000	–	15.000	–	15.000
Rice husk	–	13.000	–	13.500	–	13.500
Total	100	100	100	100	100	100

P1 = 2–6 weeks of age, P2 = 8–12 weeks of age, P3 = 14–18 weeks of age, L7 = diet with a high protein content and excessive amino acids, but with minimally 20% deficiency of digestible Lys in relation to the level of the other amino acids, N-Free = diet free from nitrogen and amino acids with adequate requirements of the other nutrients and AME_N

[1]content/kg: folic acid 0.95 g, pantothenic acid 12 g, niacin 38 g, biotin 0.060 g, vitamin A 8 000 000 UI, vitamin B1 2.4 g, vitamin B12 0.012 g, vitamin B2 6 g, vitamin B6 2.5 g, vitamin D3 2 300 000 UI, vitamin E 12 350 UI, vitamin K3 1.8 g, Se 0.3 g, antioxidant 0.250 g, excipient quantity sufficient to 100 g

[2]content/kg: Mn 20 g, Fe 100 g, Zn 160 g, Cu 16 g, I 1.5 g, excipient quantity sufficient to 1000 g

Average initial body weight (± standard error (SE)) was 110.73 ± 0.01 g and in breeding and rearing phases birds with average body weight (± SE) of 549.21 ± 0.16 g and 1028.98 ± 0.24 g were used, respectively, which was in accord with the range of body weight recommended by the strain guide for the period (+/–5% of the standard weight) (Granja Planalto, 2005).

The experiments were conducted in a digestibility facility equipped with a negative-pressure ventilation system and automated temperature control. The birds were housed in cages equipped with feeders and a line of nipple drinkers. The experimental design was completely randomized, with eight treatments and six replicates of 15 pullets per experimental unit in the initial phase and 8 pullets for the other phases. Different birds were used in each trial, and fewer pullets were used in the later phases compared with the initial phase due to the capacity of the installations used for each phase and the need to maintain an adequate density of birds in each experimental unit.

The diets comprised corn, soybean meal, and crystalline amino acids and contained Lys as the first limiting amino acid. Two diets for each experiment were prepared to meet the minimum amounts of nutrients and apparent metabolizable energy (AME_N) as recommended by Rostagno et al. (2005), except for protein and amino acids. The first diet had a high protein content and an excess of amino acids but minimally a 20% deficiency of digestible Lys compared to other amino acid levels. The second diet was nitrogen-free and was formulated to meet the requirements of all other nutrients and the AME_N, except for amino acids and protein (Table 1).

The experimental digestible Lys levels were defined according to Fisher and Morris' (1970) procedure by successive diluting the high protein content diet with the nitrogen-free diet. This calculated amino acid level should provide approximately 1.2 times more digestible lysine than recommended by Rostagno et al. (2005). All other amino acid requirements were calculated in a similar manner, where the high protein diets (L7) provided at least 1.4 multiple of these requirements, thus ensuring that the amino acid tested was limiting in all diets in the dilution series. The proportion of the dietary L7 in each treatment is listed in Tables 2 and 3.

The treatments consisted of increasing digestible Lys levels, from L1 to L7. The eighth level was added to confirm that the responses were due to the first limiting amino acid (Lys). Thus, in the 2–6-, 8–12-, and 14–18-week-old animals, the eighth treatment was prepared by adding a small quantity of L-lysine HCl (78%) to the L1 diet, so the L8 diet had the same digestible Lys level as the L2 diet.

The diet formulations were adjusted based on the compositions and digestibility coefficients of the amino acids in the ingredients (corn, wheat bran, corn gluten meal 60%, and soybean meal). The precision-fed assay was conducted using cecec-

Table 2. Digestible amino acid composition analyzed (%)

Items	P1		P2		P3	
	L7	AA/Lys	L7	AA/Lys	L7	AA/Lys
Crude protein	21.625		16.998		14.549	
Methionine + cystine	0.909	85	0.698	93	0.552	95
Methionine	0.606	57	0.445	59	0.316	55
Lysine	1.067	100	0.748	100	0.578	100
Tryptophan	0.239	22	0.179	24	0.148	25
Threonine	0.840	79	0.591	79	0.465	81
Arginine	1.353	127	1.004	134	0.754	130
Valine	0.966	90	0.725	97	0.612	106
Isoleucine	0.864	81	0.656	87	0.519	90
Leucine	1.809	170	1.474	197	1.536	265
Phenylalanine	1.021	97	0.772	103	0.678	117

P1 = 2–6 weeks of age, P2 = 8–12 weeks of age, P3 = 14–18 weeks of age, L7 = diet with a high protein content and excessive amino acids, but with minimally 20% deficiency of digestible Lys in relation to the level of the other amino acids, AA = amino acids, Lys = lysine

Table 3. Proportion (%) of the high protein diet (L7) in the composition of the treatments in three experimental phases

Level	P1			P2			P3		
	L7	DFAA	Lys	L7	DFAA	Lys	L7	DFAA	Lys
L1	30	70	0.320	30	70	0.224	30	70	0.173
L2	40	60	0.427	40	60	0.299	40	60	0.231
L3	50	50	0.534	50	50	0.374	50	50	0.289
L4	60	40	0.640	60	40	0.449	60	40	0.347
L5	70	30	0.747	70	30	0.524	70	30	0.405
L6	90	10	0.960	90	20	0.673	90	20	0.520
L7	100	0	1.067	100	0	0.748	100	0	0.578

P1 = 2–6 weeks of age, P2 = 8–12 weeks of age, P3 = 14–18 weeks of age, Lys = lysine, DFAA = diet free of amino acid

tomized Leghorn roosters (Sibbald, 1987). After 24 h of feed withdrawal, six cecectomized roosters (approximately 40 weeks old) were tube-fed approximately 30 g of each of the three samples (corn, wheat bran, and corn gluten meal 60%, and soybean meal). Excreta were then quantitatively collected for 48 h, freeze-dried, weighed, and ground for later analysis. Endogenous corrections for amino acids were made using six roosters that had been fasted for 48 h. The amino acid compositions of the ingredients, whole body, diet, and excreta were analyzed using acid hydrolysis of proteins under acidic conditions at 110°C for 23 h after performing acid oxidation of methionine and cystine. The hydrolysates were adjusted to a pH of 2.20 and the amino acids were separated by ion exchange chromatography and then determined using photometric detection at 570 nm after reaction with ninhydrin. For total tryptophan, after basic hydrolysis of the proteins, the sample was hydrolyzed under alkaline conditions with barium hydroxide and heated in an autoclave at 120°C for 16 h. The hydrolysates were acidified with chlorhydric acid to pH 3.0. Tryptophan from the hydrolysates was separated by reverse phase high performance liquid chromatography (HPLC) and determined by fluorometric detection.

The feed intake (FI) and body weight gain (BWG) data were collected every two weeks. To estimate the efficiency of utilization for Lys deposition, the data of the first two weeks of each experiment (2–4-, 8–10-, and 14–16-week-old animals) were considered. The efficiency of utilization of Lys was obtained by linear regression between Lys deposition (DLys) in the whole body and Lys intake (iLys) subtracting the maintenance requirement. The correction in the Lys intake was obtained by subtracting the daily Lys intake from the Lys requirement for maintenance of 31 mg of Lys per kg BW (Siqueira et al., 2011).

Mathematical functions were applied to explain the relationship between the digestible Lys intake (iLys, mg/day) and the BWG (g/day) and feed conversion ratio (FCR, g/g). The following functions were used to fit the responses of the variables to digestible iLys: quadratic polynomial (QP), broken-line (BL), and broken-line function with quadratic ascending (BLQ).

The BL function obtained from Robbins et al. (2006) describes the relationship between the variables with digestible iLys (X) using three parameters, as demonstrated in Eq. (1):

$$BWG = BWG_{max} + U \times (R - X) \qquad (1)$$

where:
BWG_{max} = asymptotic response of the function (β1)
U = slope at the breaking point (β2)
R = requirement to express the breaking point BWG_{max} (β3)

The estimates of this function are valid if $X < R$; when $X \geq R$, then $BWG = BWG_{max}$.

The broken-line with quadratic ascending function (BLQ) was also obtained from Robbins et al. (2006). This function describes the relationship between BWG and digestible iLys (X) with three parameters and has the same biological meaning as Eq. (1).

$$BWG = BWG_{max} + U \times (R - X)^2 \qquad (2)$$

where:
BWG_{max} = asymptotic response of the function (β_1)
U = slope at the breaking point (β_2)
R = requirement to express the breaking point BWG_{max} (β_3)

The estimates of this function are valid if X < R; when X ≥ R, then BWG = BWG_{max}.

The quadratic polynomial function (QP) given in Eq. (3) can fit the increase and decrease in performance well (but not the plateau):

$$BWG = \beta_1 + \beta_2 \times X + \beta_3 \times X^2 \quad (3)$$

where:
BWG = dependent variable
X = digestible iLys
β_1, β_2, β_3 = constants

The increase and decrease in BWG are well adjusted if β_2 is positive and β_3 is negative (Pesti et al., 2009). The optimal digestible Lys intake (mg/day) was obtained by the first QP derivative ($\beta_2 + 2\beta_3 \times X = 0$).

The digestible Lys requirement was estimated using the quadratic functions by establishing the first point at which the quadratic response curve crossed the plateau (BWG_{max}) value established from the BL function, as given in Eq. (4):

$$X = \frac{-\beta_2 \pm \sqrt{\beta_2^2 - 4 \times \beta_3(\beta_1 - BWG_{max})}}{2 \times \beta_3} \quad (4)$$

The coefficient of determination (R^2) and the Akaike information criterion (AIC) were adopted to assess the best functions. The data were analyzed for error normality (Cramer-Von Mises) and variance homoscedasticity (Levene). These assumptions were met for all variables studied in the three experiments (2–6-, 8–12-, and 14–18-week-old birds). The statistical analyses were performed considering a significance level of 5% and using the SAS software (Statistical Analysis System, Version 9.1, 2009).

RESULTS AND DISCUSSION

The body weight gain (BWG) and feed conversion ratio (FCR) responses of 2–6-, 8–12-, and 14–18-week-old pullets fed increasing levels of digestible Lys are presented in Table 4.

In the first experiment, the BWG response to L-lysine HCl (78%) (level L8) indicated that lysine was the most limiting amino acid. The feed intake was lower in the birds fed the L1 diet (3.20 g/kg), mainly in 2–6-week-old animals. The BWG responses to the lowest level (L1) treatment relative to the maximum BWG (L6) of each phase were 28% (2–6-week-old birds), 58% (8–12-week-old birds), and 44% (14–18-week-old birds), with an average value of 43%. According to Bowmaker and Gous (1991), it is important for the bird to avoid the effects of an unbalanced energy intake that may occur when the bird increases its intake of feed with a lower concentration of amino acids. The most diluted diets had energy concentrations similar to those of the L7 diet despite their lower amino acid concentrations, so the amount of energy consumed by the birds fed these diets far exceeded the amount required for BWG, considering the low gain in birds fed the L1 diet (Bowmaker and Gous, 1991). The dilution of the amino acid levels described above affected the feed conversion ratio.

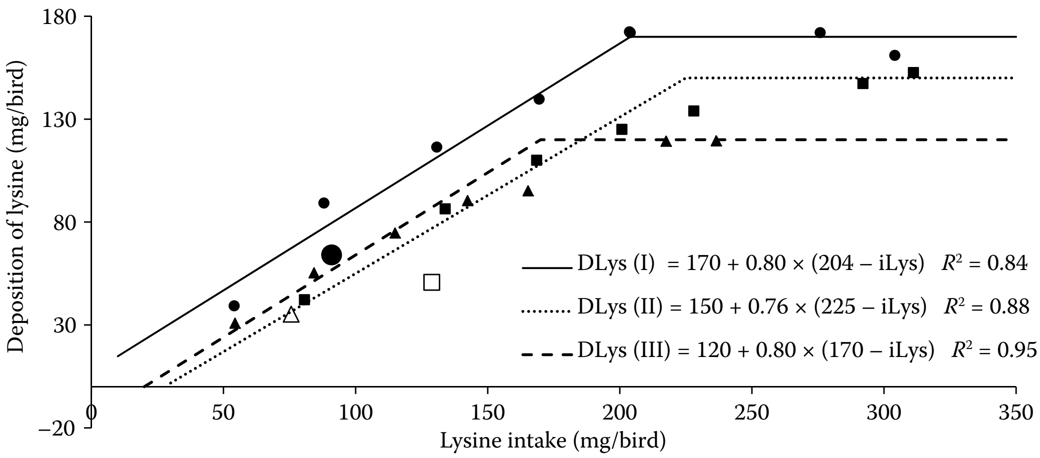

Figure 1. Graphical representation of the efficiencies of utilization of lysine for deposition in the phases of 2–4 (I), 8–10 (II), and 14–16 (III) weeks of age

(●) mean responses of the birds to L1–L7 diets (2–4 weeks of age), (■) mean responses of the birds to L1–L7 diets (8–10 weeks of age), (▲) mean responses of the birds to L1–L7 diets (14–16 weeks of age), (○) response of the birds to L8 diet (2–4 weeks of age), (□) response of the birds to L8 diet (8–10 weeks of age), (Δ) response of the birds to L8 diet (14–16 weeks of age)

Table 4. Feed intake (FI), lysine intake (iLys), body weight gain (BWG), and feed conversion ratio (FCR)

	Level Lys (%)	FI (g/day)	iLys (mg/day)	BWG (g/day)	FCR (g/g)
2–6 weeks of age (n = 42)					
L1	0.320	17.40	57.53	2.83	6.15
L2	0.426	19.58	86.31	4.36	4.74
L3	0.533	20.38	112.32	6.74	3.02
L4	0.641	21.19	140.37	8.35	2.54
L5	0.747	21.24	164.09	8.90	2.39
L6	0.960	22.06	219.04	10.02	2.20
L7	1.067	21.87	241.29	9.98	2.19
L8	0.426	19.07	84.09	3.30	5.78
$P > F$		< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV (%)		3.51	3.28	4.66	6.47
8–12 weeks of age (n = 42)					
L1	0.224	48.01	111.86	7.12	6.93
L2	0.299	49.99	155.04	7.68	6.60
L3	0.374	50.39	195.69	9.06	5.56
L4	0.449	51.13	238.62	10.74	4.77
L5	0.524	51.54	280.6	11.99	4.30
L6	0.673	51.64	361.32	12.25	4.22
L7	0.748	51.42	399.7	12.01	4.28
L8	0.299	50.07	155.56	7.47	6.72
$P > F$		0.1124	< 0.0001	< 0.0001	< 0.0001
CV (%)		4.59	4.69	3.38	5.63
14–18 weeks of age (n = 42)					
L1	0.173	52.30	96.38	4.56	11.54
L2	0.231	52.97	130.17	5.14	10.31
L3	0.289	56.36	173.13	7.06	8.07
L4	0.347	57.00	210.45	8.63	6.62
L5	0.405	58.27	250.95	9.48	6.15
L6	0.52	58.99	326.50	10.48	5.63
L7	0.578	57.67	354.65	9.70	5.95
L8	0.231	52.92	130.04	5.11	10.37
$P > F$		< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV (%)		2.44	2.52	5.32	7.5

P = value of associated probability, F = F-test value, CV = coefficient of variation

The L1 pullets displayed approximately 2.15 times greater FCR than the L7 pullets (Table 4).

Figure 1 shows the iLys and DLys for each treatment at the phases of 2–4-, 8–10-, and 14–16-week-old animals. The increase in iLys promoted an increase in DLys, hence efficiency of utilization, in the three phases evaluated. The efficiencies were estimated at 80% (2–4-week-old birds), 76% (8–10-week-old birds), and 80% (14–16-week-old birds).

The efficiencies of utilization of Lys observed in this study (80, 76, and 80%) were obtained with responses within the range where Lys was limiting and linearly related to protein deposition. To our knowledge, however, there have been no other studies determining efficiency in pullets. Efficiency, as observed here, was higher than that previously observed for birds of layer genotype (Fatufe et al., 2004), in which the efficiency of Lys utilization showed maxima of 74%. These maxima were achieved at intakes which were much lower than those needed for high protein deposition.

For 2–6-week-old pullets, the BWG and FCR responses to the digestible Lys intake were well fitted by all three functions (Figure 2).

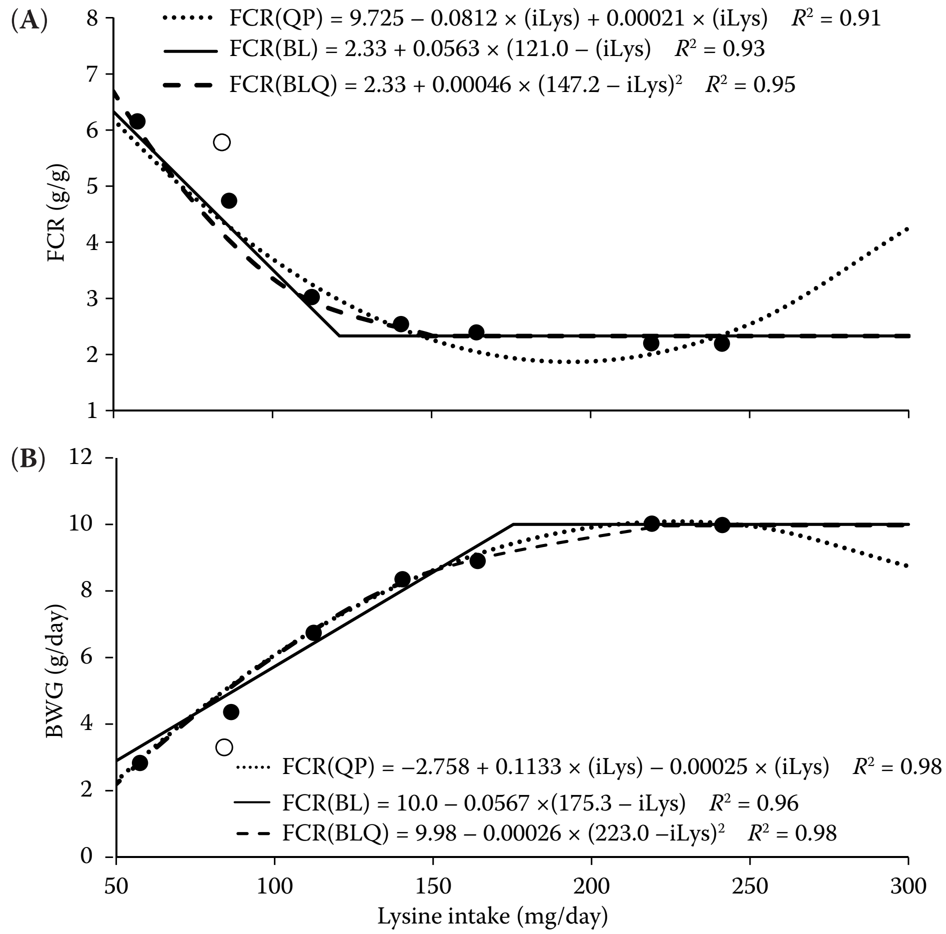

Figure 2. Graphical representation of quadratic polynomial (QP), broken-line (BL), and broken-line with quadratic ascending (BLQ) functions for (**A**) feed conversion ratio (FCR) and (**B**) body weight gain (BWG) responses of 2–6-week-old pullets receiving gradual levels of digestible lysine

(○) response of the birds to L8 diet (2–6 weeks of age, (●) response of the birds to L1–L7 diets (2–6 weeks of age)

The BL function predicted a breaking point (β_3) for BWG and FCR at 175 and 121 mg/day Lys, respectively (Table 5). BLQ predicted a breaking point (β_3) for BWG and FCR at 223 and 147 mg/day Lys, respectively (Table 5). The estimated daily digestible Lys intakes obtained by the first derivative of the QP function were 227 and 193 mg/day for BWG and FCR, respectively. The first intercepts of the quadratic curve (Eq. (4)) with the broken-line plateau calculated for BWG and FCR occurred at 202 and 146 mg/day, respectively.

In the second phase (8–12 weeks of age), the pullet BWG and FCR responded to gradual digestible Lys level increases (Figure 3).

The BL function predicted a breaking point for BWG at 287 and for FCR at 269 mg/day Lys. The digestible Lys intakes required to achieve BWG_{max} as predicted by the BLQ and QP functions were similar for BWG and FCR, estimated at 390 and 358 mg/day, respectively. The first intercepts of the quadratic curve (Eq. (4)) with the broken-line plateau calculated for the BWG and FCR occurred at 338 and 312 mg/day, respectively.

The relationships between BWG and FCR and the digestible Lys intake were estimated by fitting the BL, BLQ, and QP functions for pullets from 14 to 18 weeks of age (Figure 4).

The minimum digestible Lys intake determined by BL was 255 for BWG and 226 mg/day for FCR. The QP function of the digestible Lys intake (Table 5) exhibited maximum BWG (i.e. upper asymptote) and minimum FCR (i.e. lower asymptote) at 337 and 308 mg/day, respectively. The first intercepts of the quadratic curve (Eq. (4)) with the broken-line plateau calculated for BWG and FCR occurred at 300 and 259 mg/day, respectively.

The results demonstrated that the digestible Lys intake calculated for optimum FCR was consid-

Table 5. Estimates of the optimum lysine intake by different mathematical models in the phases 2–6, 8–12, and 14–16 weeks of age

Models	Response variable	β_1	β_2	β_3	R^2	AIC	Lys
2–6 weeks of age							
QP	BWG	–2.75792	0.11334	–0.00025	0.98	–81.8	226.7
BL	BWG	10.0	–0.0567	175.3	0.96	–54.9	175.3
BLQ	BWG	9.98	–0.00026	223.0	0.98	–82.3	223.0
BL + QP[1]	BWG	–	–	–	–	–	202.2
QP	FCR	9.72540	–0.08124	0.00021	0.91	–73.8	193.4
BL	FCR	2.33	0.0563	121.0	0.93	–82.5	121.0
BLQ	FCR	2.33	0.00046	147.2	0.95	–98.7	147.2
BL + QP	FCR						146.3
8–12 weeks of age							
QP	BWG	1.36537	0.05611	–0.000072	0.93	–46.6	389.7
BL	BWG	12.12	–0.0302	286.6	0.95	–64.6	286.6
BLQ	BWG	12.25	–0.00007	390.1	0.93	–46.4	390.1
BL + QP	BWG	–	–	–	–	–	338.1
QP	FCR	10.33762	–0.03443	0.000048	0.88	–78.5	358.6
BL	FCR	4.28	0.0174	268.9	0.91	–87.7	268.9
BLQ	FCR	4.22	0.00005	357.6	0.88	–77.5	357.6
BL + QP	FCR	–	–	–	–		311.9
14–18 weeks of age							
QP	BWG	–1.62229	0.069495	–0.000103	0.94	–47.1	337.4
BL	BWG	9.97	–0.0356	254.70	0.95	–51.0	254.7
BLQ	BWG	10.12	–0.0001	341.2	0.94	–46.7	341.2
BL + QP	BWG	–	–	–	–	–	300.0
QP	FCR	18.57534	–0.08436	0.000137	0.93	–36.1	307.9
BL	FCR	5.91	0.0436	226.4	0.93	–37.6	226.4
BLQ	FCR	5.77	0.00014	299.1	0.92	–35.0	299.1
BL + QP	FCR	–	–	–	–	–	259.1

BL = broken-line function, QP = quadratic polynomial function, BLQ = broken-line function with quadratic ascending, BWG = body weight gain, FCR = feed conversion ratio, β_1, β_2, β_3 = constants, R^2 = coefficient of determination, AIC = Akaike information criterion, Lys = lysine

[1]the estimate of Lys requirements by quadratic models was determined by establishing the first point at which the quadratic response curve crossed the plateau value established from the BL model

erably lower than that determined for BWG_{max}, which can be attributed to the formulation technique used. According to Gous and Morris (1985), when the diet is marginally deficient in Lys, the birds increase their FI, most likely in an attempt to ingest sufficient Lys to meet their metabolic demands.

The best fits (R^2) were observed for BWG. For 2–6-week-old pullets, based on the AIC, the BLQ function performed the best, regardless of the studied variable. In this function, the response was curvilinear as the requirement was approached (Pesti et al., 2009). BLQ considers no response above the plateau and therefore corrects the bilateral symmetry assumed by the QP function. We expected to achieve similar estimates for the first intercept of the QP curve with the BL and BLQ plateaus, but a procedural similarity was observed only for FCR.

In the other phases (8–12 and 14–18 weeks of age), the BL function performed the best. The upper (BWG_{max}, β_1) and lower (FCR_{min}, β_1) asymptotes estimated by BL and BLQ were similar; however, the estimated optimal digestible Lys intake values were significantly different (Table 5). The quadratic fit does not establish objective breaking points (Baker et al., 2002). Estimating amino acid requirements using broken-line regression analysis establishes requirements objectively based on the inflection point of the fitted response (Baker et al., 2002; Robbins et al., 2006). Baker et al. (2002) demonstrated that the BL breaking point estimated the minimum iLys value, which is advantageous for calculating amino acid ratios. The same cannot be achieved using the BLQ function, as its estimate is similar to those obtained using quadratic regression.

For the BWG first intercept (iLys value), the QP curve with the BL plateau estimated a requirement value close to that predicted at 89, 87, and 89% of the upper asymptote values of the quadratic equation for 2–6-, 8–12-, and 14–18-week-old pullets, respectively. Fatufe et al. (2004) demonstrated that pullets (Lohmann White) displayed stable responses at 179 mg Lys/day in the diet during the starter phase. The average requirement of 202 ((223 + 179)/2) mg Lys/day reported by Halle (2002) and Fatufe et al. (2004)) was similar to that determined in the present work. The recommendation of the hybrid producer (Granja Planalto, 2005) is 195 mg Lys/day in the initial phase and 344 mg Lys/day in the breeding phase and these values are in agreement with the results of this study, with a difference of 7 and 6 mg of Lys, respectively. However, for the rearing phase the recommendation of 467 mg Lys/day is higher than the estimated value of 300 mg Lys/day from this study, to optimize BWG.

Rodrigueiro et al. (2007) indicated that an intake of 204 mg Lys/day could not stabilize the BWG responses, and the population BWG_{max} was not described in the study. In the study of Jardim

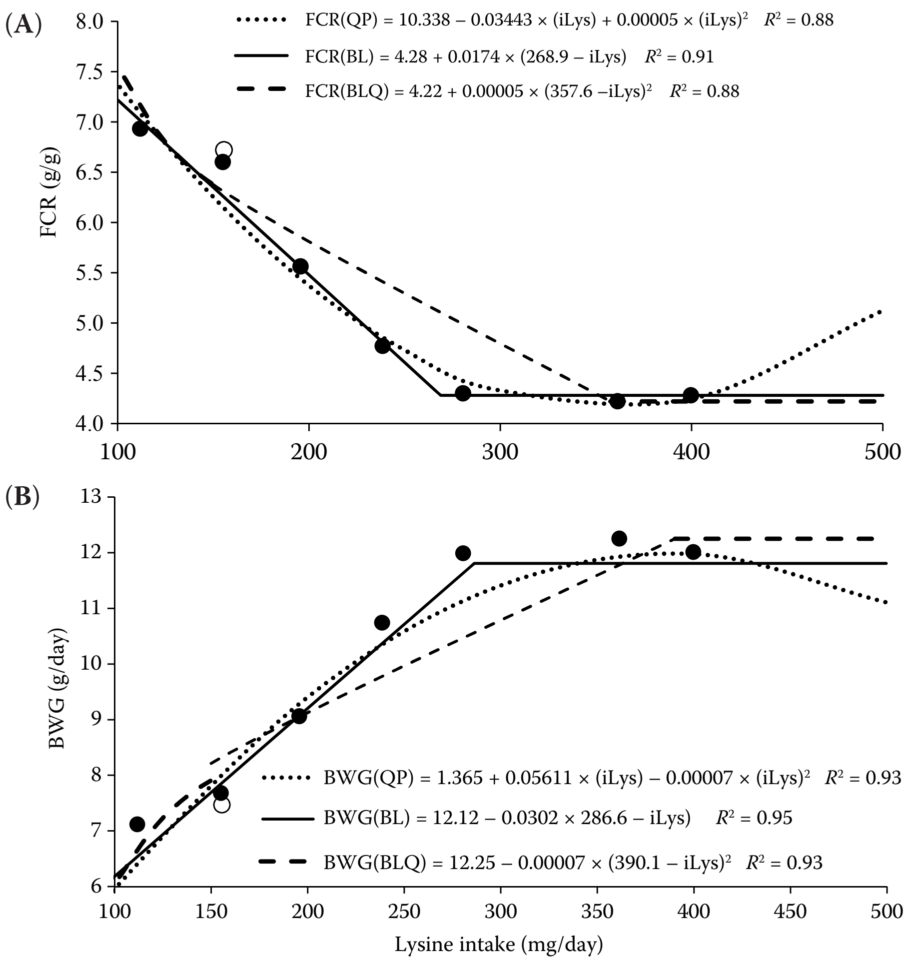

Figure 3. Graphical representation of quadratic polynomial (QP), broken-line (BL), and broken-line with quadratic ascending (BLQ) functions for (**A**) feed conversion ratio (FCR) and (**B**) body weight gain (BWG) responses of 8–12-week-old pullets receiving gradual levels of digestible lysine

(○) response of the birds to L8 diet (8–12 weeks of age), (●) response of the birds to L1–L7 diets (8–12 weeks of age)

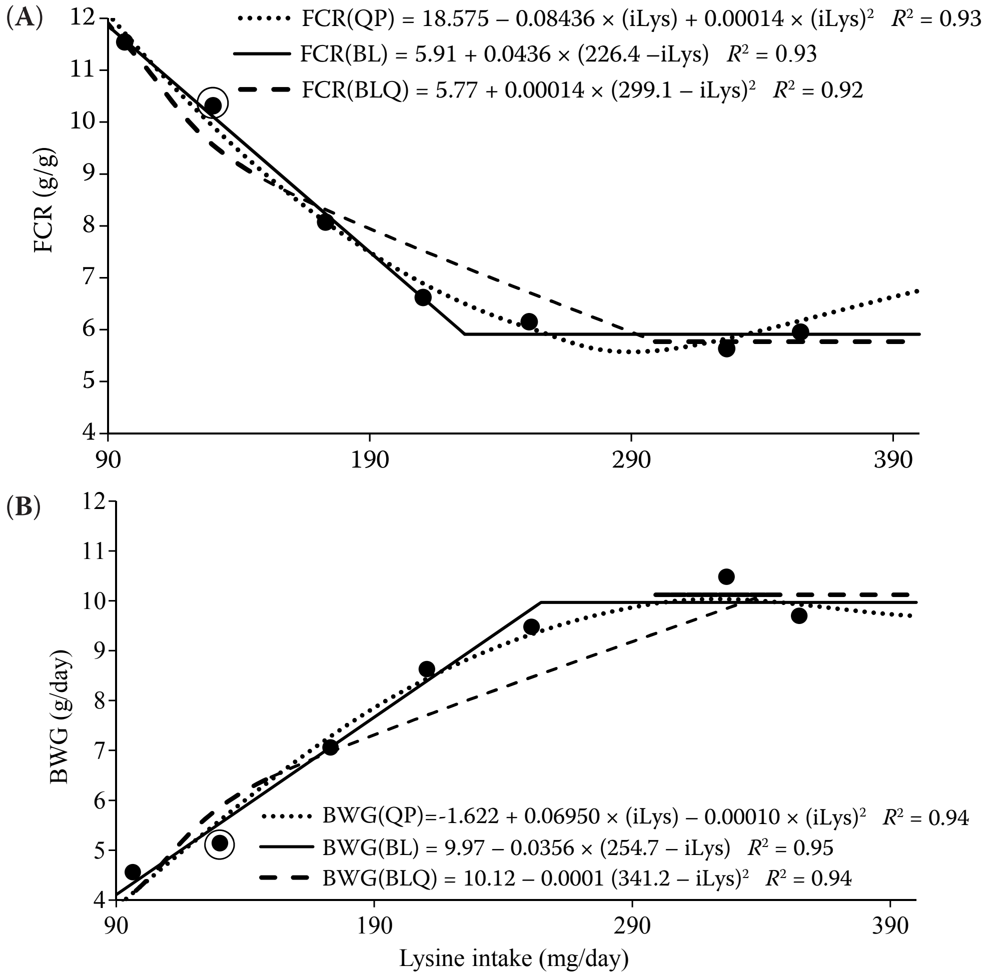

Figure 4. Graphical representation of quadratic polynomial (QP), broken-line (BL), and broken-line with quadratic ascending (BLQ) functions for (**A**) feed conversion ratio (FCR) and (**B**) body weight gain (BWG) responses of 8–18-week-old pullets receiving gradual levels of digestible lysine

(○) response of the birds to L8 diet (14–18 weeks of age), (●) response of the birds to L1–L7 diets (14–18 weeks of age)

Filho et al. (2011), the lysine levels did not influence the BWG and FI responses in the pre-laying (16–20-week-old) and pre-peak (21–25-week-old) Lohmann White birds and they recommend 402 mg Lys/day or 0.6% of Lys/kg of feed.

Accordingly, the calculation of the response limits of the population is crucial in order to identify the physiological mechanisms, apart from subsidising criteria, that affect the determination of the desired productive level or the digestible Lys supply that will adequately supply a certain proportion of the population.

The optimal digestible lysine intake was 338 to 300 mg/day for 8–12- and 14–18-week-old pullets, respectively. In these phases, the intakes equivalent to the average BWG intercepts of the quadratic curve were 328 and 311 mg/day as reported by Silva et al. (2000a) and Silva et al. (2000b), respectively.

In general, the results obtained in this study indicate that the optimal digestible iLys intake estimated depends on the mathematical function used. Modifying the digestible Lys concentration in the diet is a practical way to achieve amino acid requirements; however, it is influenced by FI, and thus a precise reference is essential to determine the ideal amino acid concentration.

CONCLUSION

The association of broken line and quadratic response curves enables to take advantage of both procedures to determine a more optimized recommendation for a better performance. Thus, the recommendation of digestible lysine intakes to optimize the body weight gain and feed conversion ratio responses of Dekalb white pullets were 202, 338, and 300 and 146, 312, and 259 mg/day for 2–6-, 8–12-, and 14–18-week-old animals, respectively.

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Corresponding Author

Prof. Dr. Nilva Kazue Sakomura, São Paulo State University (UNESP), Faculty of Agrarian and Veterinary Sciences of Jaboticabal, Department of Animal Sciences, Via de Acesso Prof. Paulo Donato Castellane s/n, 14883-900 Jaboticabal, São Paulo, Brazil
Phone: +551 632 092 682, e-mail: sakomura@fcav.unesp.br