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Reevaluation of the digestible lysine requirement for broilers based on genetic potential

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ABSTRACT: Broiler strains available in the poultry industry present different requirements for dietary lysine due to their different growth potentials as a result of their genetic makeup. This study aimed to determine the model parameters for maximum nitrogen retention ($NR_{max}T$), the nitrogen maintenance requirement (NMR) and the efficiency of lysine utilization (bc^{-1}) to reevaluate the lysine (Lys) requirements of male and female broilers. Nitrogen balance trials were performed during three periods (I: 6-21 days, II: 22-37 days, and III: 38-53 days). Seven treatments were used for males and females; the treatments consisted of seven diets with protein levels ranging from 61 to 364 g kg^{-1} dry matter, with Lys being limiting in the dietary nitrogen (4.91 g of Lys in 16 g of N). Nitrogen intake (NI), excretion (NEX), deposition (ND, ND=NI-NEX) and retention (NR, NR=ND+NMR) values were obtained. The NMR was represented by the exponential relationship between NEX and NI. The $NR_{max}T$ and bc^{-1} were estimated by the exponential fit between ND and NI. The $NR_{max}T$, bc^{-1}, and NMR values were combined in a model to estimate Lys intake by simulating different percentages of the $NR_{max}T$. The Lys intake estimates were 581, 1,538, and 2,171 mg day^{-1} for males and 512, 1,340, and 1674 mg day^{-1} for females during periods I, II, and III, respectively. Due to the flexibility of the model, it is possible to calculate the Lys intake for percentages of NR in the range of practical performance data.

Keywords: amino acid, exponential model, maintenance, nitrogen retention, performance

Introduction

The broiler strains available in the poultry market present different dietary amino acid requirements due to the different growth potentials of each bird, which are determined mainly by genotype (Smith and Pesti, 1998) and other factors, such as sex and age (Zuprizal et al., 1992; Rosa et al., 2001). Accordingly, the determination of the amino acid requirements of poultry is dependent on adequate description of the potential of the birds for protein deposition according to genotype, age and sex. This potential has been determined in nitrogen balance trials (Samadi and Liebert, 2007).

In addition to the maximum potential for protein deposition, the efficiency of amino acid utilization and nitrogen requirements for maintenance have been integrated into a factorial equation that enables amino acid requirements to be estimated (Samadi and Liebert, 2007). The efficiency of amino acid utilization typically has been represented by a model, in which the response of the bird to amino acid intake is constant and linear to the point where maximum protein deposition is achieved. However, studies have demonstrated that the response of a group of birds to nutrient intake is curvilinear due to variability in maintenance and growth potential (Curnow, 1973).

Broilers with a high capacity for lean deposition require higher amounts of lysine to maximize both their performance and the protein deposition rate in the carcass. Lysine is considered to be the second most limiting amino acid after the sulfur-containing amino acids for broilers fed diets based on corn and soybean meal. For these reasons lysine was chosen as the reference amino acid as the ideal protein concept, in which all other essential amino acids are formulated into the diet as a ratio to lysine (Emmert and Baker, 1997). Consequently, obtaining an accurate and precise estimate of lysine requirements of broilers is highly desirable. In addition, formulating diets based on an ideal protein concept results in an efficient use of dietary protein by improving nitrogen utilization, resulting in minimal nitrogen excretion.

Responses to dietary lysine levels have been studied for decades and mathematical models have been developed to predict lysine requirements (Han and Baker, 1994; Samadi and Liebert, 2007; Samadi and Liebert, 2008; Goulart et al., 2008). However, the daily dietary lysine requirements have changed over the past few years because of the considerable influence of progress in genetics. Thus, this study aimed to reevaluate the digestible lysine requirements of male and female broilers based on their genetic potential.

Materials and Methods

Birds and housing

The experiment was conducted in Jaboticabal, in the state of São Paulo, Brazil (21º15'16" S; 48º19'19" W, altitude 607 m). Nitrogen balance trials were performed in three periods (I: 6-21 days, II: 22-37 days, and III: 38-53 days) using 84 Cobb500 genotype broilers in each period (42 males and 42 females). The birds were individually distributed in a completely randomized design with seven treatments composed of six males and six females per experimental unit. The experimental units consisted of metabolic cages with wire flooring equipped with individual feeders and water drinkers. This study was approved by the Ethics Committee on Animal Use (CEUA) of the Faculty of Agriculture and Veterinary Sciences, UNESP, Jaboticabal (protocol number 007125-08).

Experimental diets

The first six treatments consisted of varying levels of dietary protein at 61 (N1), 124 (N2), 183 (N3), 239 (N4), 295 (N5), and 364 (N6) g kg^{-1} of dry matter (DM); these treatments all maintained lysine as the first limiting amino acid (at 4.91 g of lysine for every 16 g of N). The seventh treatment (N7) was obtained by adding 4 g of L-lysine HCl (78 %) per kg of feed to a diet composition similar to that of N1 containing 61 g kg^{-1} DM to verify that lysine was the first limiting amino acid in the diets. The experimental diets were obtained by diluting a high protein diet (consisting of soybean meal, corn gluten meal and crystalline amino acids) with a protein-free diet consisting mainly of corn starch (Table 1) to obtain the graded levels of dietary protein and to keep lysine as the first limiting amino acid in all diets (thereby maintaining the amino acid ratio). The composition of digestible amino acids analyzed in the experimental diets is presented in Table 2. The proportion of high protein diet (N6) to protein-free diet used in the preparation of the experimental diets was 15:85 for N1, 32:68 for N2, 49:51 for N3, 66:34 for N4, 83:17 for N5, and 100:0 for N6.

Feeding and data collection

The experimental period was divided into five days of adaptation to the diets followed by two consecutive periods of excreta collection (five days each); at the end of each period, nitrogen balance data were obtained from six different birds receiving the same diet. The experimental diets were provided *ad libitum* throughout the entire study period. During the collection periods,

Table 1 – Composition of experimental diets.

Ingredients	Diets	
(g kg^{-1})	High protein (N6)	Protein-free
Soybean meal (45 %)	540.3	-
Corn	170.0	-
Corn gluten meal (60 %)	165.0	-
Soybean oil	80.7	65.0
Dicalcium phosphate	19.0	27.0
Limestone	7.2	3.4
Salt	5.1	5.2
DL-Methionine (99 %)	4.5	-
L-Lysine HCl (78 %)	3.5	-
L-Threonine (99 %)	1.5	-
Vitaminic supplement[1]	1.0	1.0
Choline chloride (70 %)	0.7	0.7
Mineral supplement[2]	1.0	1.0
Anticoccidial[3]	0.5	0.5
Potassium chloride	-	12.8
Corn starch	-	595.0
Inert (sand)	-	59.6
Rice husk	-	128.8
Sugar	-	100.0

Nutritional composition (g kg^{-1} DM)[4]	Diets						
	N1	N2	N3	N4	N5	N6	N7
Crude protein	61.0	124.0	183.0	239.0	295.0	364.0	61.0
Ether extract	72.1	89.1	83.2	88.7	94.3	99.8	72.1
Crude fiber	48.4	45.5	42.6	39.7	36.8	33.9	48.4
Calcium	9.3	9.3	9.3	9.3	9.3	9.3	9.3
Sodium	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Potassium	6.6	7.4	8.2	9.0	9.8	10.6	6.6
Available phosphorus	4.7	4.7	4.7	4.7	4.7	4.7	4.7
AME_n (Mcal kg^{-1})[5]	3.15	3.15	3.15	3.15	3.15	3.15	3.15

[1]Vitaminic mixture (content per kg of the supplement): Vitamin A, 7,000,000 IU; Vitamin D3, 2,200,000 IU; Vitamin E, 11,000 mg; Vitamin K3, 1,600 mg; Vitamin B1, 2,000 mg; Vitamin B2, 5,000 mg, Vitamin B12, 12,000 µg; Niacin, 35,000 mg; Pantothenic acid, 13,000 mg; Folic acid, 800 mg; Vehicle q.s.p. 1,000 g; [2]Mineral mixture (content per kg of the supplement): Iron, 10,000 mg; Copper, 16,000 mg; Iodine, 2,400 mg; Zinc, 100,000 mg; Manganese, 140,000 mg; Selenium, 400 mg; Vehicle q.s.p. 1,000 g; [3]Coxistac 12 %; [4]Calculated composition using data from the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011); [5]Apparent metabolizable energy corrected by nitrogen balance, calculated.

Table 2 – Digestible amino acid content of experimental diets.

Amino acids (AA)[1]	N1	N2	N3	N4	N5	N6	N7	AA-Ratio (AA/lysine)
Lysine	4.53	4.74	4.92	5.07	5.16	5.04	9.43(100)[2]	100
Arginine	5.18	5.43	5.63	5.80	5.90	5.77	4.87(52)	114
Histidine	2.07	2.17	2.25	2.32	2.36	2.31	1.95(21)	46
Isoleucine	3.64	3.82	3.96	4.08	4.15	4.06	3.42(36)	81
Leucine	8.84	9.27	9.61	9.90	10.07	9.84	8.30(88)	195
Met+Cys	3.65	3.83	3.97	4.09	4.16	4.07	3.43(36)	81
Methionine	2.50	2.63	2.72	2.80	2.85	2.79	2.35(25)	55
Threonine	3.36	3.52	3.65	3.76	3.82	3.73	3.15(33)	74
Tryptophan	0.88	0.92	0.96	0.99	1.00	0.98	0.83(9)	19
Valine	3.83	4.01	4.16	4.29	4.36	4.26	3.60(38)	85

[1]Analyzed composition of the amino acids in experimental diets (g of AA in 16 g of N); [2]AA ratio in counter-proof diet.

the excreta were collected in trays (free from feathers) and weighed at the end of each period.

Chemical analysis

The samples of excreta obtained from each experimental unit during each period were thawed, homogenized, weighed in Petri dishes and frozen again. Afterwards, the excreta samples were freeze-dried for 72 h at -80 °C and 0.08 MPa of pressure. The samples were weighed to quantify the dry matter content and were then ground in a Micro Mill. The total nitrogen contents of the diets and excreta were analyzed in a nitrogen distiller using the Kjeldahl method (Method No. 2001.11) according to AOAC (2005). A factor of 6.25 was used in the conversion of the nitrogen value to crude protein (CP). The total amino acid content of the ingredients in the experimental diets was analyzed using high performance liquid chromatography (HPLC), and these values were corrected for digestible amino acids using the tabulated coefficients of digestibility (Rostagno et al., 2011).

Statistical analysis

Data were analyzed by a one-way ANOVA using a GLM procedure and were fitted to exponential models using PROC NLIN in SAS (Statistical Analysis System, version 9.1); the Levenberg-Marquardt algorithm was used to converge on a solution for these models.

Regression analysis between nitrogen intake (NI, mg $BW^{0.67}$ kg^{-1} day^{-1}) and nitrogen excretion (NEX, mg $BW^{0.67}$ kg^{-1} day^{-1}) was used in the fitting of the exponential function:

$$\mathrm{NEX} = \mathrm{NMR} \times e^{b \times \mathrm{NI}} \tag{1}$$

where NMR is the nitrogen requirement for maintenance (mg $BW^{0.67}$ kg^{-1} day^{-1}), *b* is the slope of the exponential curve and *e* is the base number of the natural logarithm (*ln*). From the exponential function (1), the NMR was estimated by considering the intercept of the curve on the y-axis (NEX) when NI = 0. The nitrogen balance or nitrogen deposition (ND, mg $BW^{0.67}$ kg^{-1} day^{-1}) was calculated as the difference between NI and NEX (ND = NI-NEX).

Regression analysis between NI and nitrogen retention (NR = ND + NMR, mg $BW^{0.67}$ kg^{-1} day^{-1}) was performed to fit another exponential model:

$$\mathrm{NR} = \mathrm{NR_{max}T} \times (1 - e^{-b \times \mathrm{NI}}) \tag{2}$$

where $NR_{max}T$ is the theoretical maximum nitrogen retention (mg $BW^{0.67}$ kg^{-1} day^{-1}). Another way to express the exponential model (2), according to Samadi and Liebert (2007), is by regression analysis between NI and ND, considering ND = NR-NMR, as shown here:

$$\mathrm{ND} = \mathrm{NR_{max}T} \times (1 - e^{-b \times \mathrm{NI}}) - \mathrm{NMR} \tag{3}$$

The model generated in equation (3) was used to estimate $NR_{max}T$ for each age, where *b* is the slope of the nitrogen retention curve and *e* is the base number of the natural logarithm (*ln*). The $NR_{max}T$ was estimated by a statistical procedure following several iterations by the Levenberg-Marquardt algorithm until the sum of the squares of the residual was minimized. The theoretical maximum nitrogen deposition ($ND_{max}T$, mg $BW^{0.67}$ kg^{-1} day^{-1}) for each period was obtained by subtracting the NMR from the $NR_{max}T$ ($ND_{max}T = NR_{max}T$ - NMR) expression.

To estimate digestible lysine requirements, the first step was to modify equation (2) by logarithmization and by several conversions, the *b* parameter, used as feed protein evaluation, can be calculated by following the equation proposed by Samadi and Liebert (2007):

$$\mathrm{b} = [ln\mathrm{NR_{max}T} - ln(\mathrm{NR_{max}T} - \mathrm{NR})] / \mathrm{NI} \tag{4}$$

The *b* value ($\times 10^{-6}$) calculated in this way and divided by the concentration of the limiting amino acid in the feed protein, *c* (g of AA in 16 g of N), was used to express the efficiency of utilization of the limiting amino acid (bc^{-1}). The bc^{-1} value ($\times 10^{-6}$) is represented by the slope of the straight line between the quality of the protein in the diet (*b*) and the limiting amino acid (LAA) concentration (*c*) in the diet provided.

The necessary NI for a defined level of NR, depending on the parameter b (feed protein quality), can be calculated from a modification of the following equation (4):

$$NI = [\ln NR_{max}T - \ln(NR_{max}T - NR)] / b \quad (5)$$

According to Samadi and Liebert (2008), the mathematical description of NI against limiting amino acid intake (LAAI) is calculated as:

$$NI = 16LAAI / c \quad (6)$$

The LAAI was obtained by replacing NI in equation (5) with equation (6), resulting in equation (7):

$$LAAI = [\ln NR_{max}T - \ln(NR_{max}T - NR)] / 16bc^{-1} \quad (7)$$

where LAAI is the intake of the LAA and bc^{-1} is the efficiency of utilization of the dietary LAA. The LAAI equation (7) was used to calculate the Lys requirement (mg day^{-1}) based on estimated NR data (targeted growth performance) and bc^{-1}. The NR was expressed as a percentage of the $NR_{max}T$ using mean values for body weights (BW, g), feed intake (FI, g day^{-1}) and Lys requirements (g kg^{-1}) from the Cobb500 management guide (Cobb, 2008; 2012) to simulate targeted growth performance recommended under practical conditions. For this simulation, the Lys requirement (mg day^{-1}) was expressed per mass of metabolic BW (mg BW$^{0.67}$ kg^{-1} day^{-1}) using a mathematical rearrangement of equation (7), where the LAAI (in this case Lys) was multiplied by $16bc^{-1}$ resulting in the following equation (7a):

$$16 \times Lys \times bc^{-1} = \ln(NR_{max}T) - \ln(NR_{max}T - NR) \quad (7a)$$

Isolating $\ln(NR_{max}T - NR)$ in equation (7a) results in equation (7b):

$$\ln(NR_{max}T - NR) = \ln(NR_{max}T) - (16 \times Lys \times bc^{-1}) \quad (7b)$$

The $\ln(x) = y$ function can be interpreted using the usual properties for inverse functions, that is $x = e^{(y)}$. Considering that $x = NR_{max}T - NR$ and $y = \ln(NR_{max}T) - (16 \times Lys \times bc^{-1})$, it is possible to derive the following equation (7c):

$$NR_{max}T - NR = e^{(\ln(NRmaxT) - (16 \times Lys \times bc^{-1}))} \quad (7c)$$

Finally, taking NR as the response in equation (7c) results in equation (8):

$$NR = NR_{max}T - e^{(\ln(NRmaxT) - (16 \times Lys \times bc^{-1}))} \quad (8)$$

From equation (8), the NR expressed as a percentage of the $NR_{max}T$ (the reference for genetic growth potential) was calculated using the $NR_{max}T$ and bc^{-1} values estimated in this study to model the Lys data and determine the Lys requirement nearest to the growth potential observed under practical conditions. The optimal Lys concentration in the diet (g kg^{-1}) was calculated from the assumptions made for the daily feed intake (± 10g) taken from the management guide for the lineage (Cobb, 2008; 2012). The optimum level of dietary lysine was calculated as the Lys requirement (g day^{-1}) divided by the feed intake (kg day^{-1}).

Results

The ND data responded ($p < 0.001$) because of the graded dietary protein supply from N1 to N6 (Table 3 and 4). Accordingly, the NEincreased ($p < 0.001$) with increasing NI and mostly exceeded the ND data observed. An increase in the ND response of birds was observed in the counter-proof treatment (N7), and this response was between the values obtained from N1 and N2, confirming that lysine was the first limiting amino acid in the experimental diets.

The NMR values obtained in this study increased from 219 mg BW$^{0.67}$ kg^{-1} day^{-1} (Period I) to 276 mg BW$^{0.67}$ kg^{-1} day^{-1} (period III) and from 225 mg BW$^{0.67}$ kg^{-1} day^{-1} (Period I) to 271 mg BW$^{0.67}$ kg^{-1} day^{-1} (period III) for males and females, respectively (Figure 1).

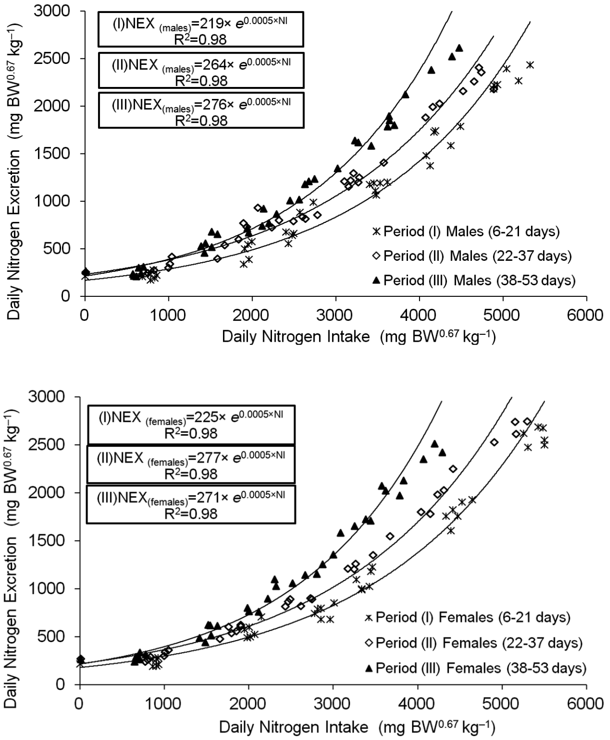

Figure 1 – Estimation of the nitrogen requirements for maintenance (NMR) by fitting an exponential function between the daily nitrogen intake (NI) and the daily nitrogen excreted (NEX) during a gradual increase in supplied protein for Cobb500 males and females. e = base of the natural logarithm (ln).

The estimated values of the ND$_{max}$T decreased, depending on age, from 3,746 mg BW$^{0.67}$ kg^{-1} day^{-1} (period I) to 2,204 mg BW$^{0.67}$ kg^{-1} day^{-1} (period III) for males and from 3,620 mg BW$^{0.67}$ kg^{-1} day^{-1} (period I) to 2,048 mg BW$^{0.67}$ kg^{-1} day^{-1} (period III) for females (Figure 2). In contrast, the *b* parameter calculated by equation (4) increased with age and was 286, 322, and 466 × 10^{-6} for males and 305, 346, and 507 × 10^{-6} for females during pe-

Table 3 – Mean body weight (BW), dry matter intake (DMI), daily nitrogen intake (NI), daily nitrogen excretion (NEX) and daily nitrogen deposition (ND) obtained in nitrogen balance trials with males receiving graded levels of protein[1].

Diets	Males								
	Period I (6-21 days)[2]								
	N1	N2	N3	N4	N5	N6	N7	RSD[3]	p-value
BW (g)	261	348	381	405	402	417	254	140	NS
DMI (g day^{-1})	32	48	45	49	48	47	40	12	NS
NI (mg BW$^{0.67}$ kg^{-1})	787	1941	2518	3493	4238	5043	1226	112	***
NEX (mg BW$^{0.67}$ kg^{-1})	225	475	741	1163	1622	2294	335	113	***
ND (mg BW$^{0.67}$ kg^{-1})	562	1466	1777	2330	2616	2750	891	93	***
Diets	Period II (22-37 days)[2]								
	N1	N2	N3	N4	N5	N6	N7	RSD[3]	p-value
BW (g)	1211	1381	1490	1579	1420	1533	1201	269	NS
DMI (g day^{-1})	105	113	111	113	106	106	114	11	NS
NI (mg BW$^{0.67}$ kg^{-1})	918	1826	2503	3193	4006	4652	1265	169	***
NEX (mg BW$^{0.67}$ kg^{-1})	324	669	812	1229	1834	2303	512	133	***
ND (mg BW$^{0.67}$ kg^{-1})	594	1157	1691	1964	2172	2349	753	87	***
Diets	Period III (38-53 days)[2]								
	N1	N2	N3	N4	N5	N6	N7	RSD[3]	p-value
BW (g)	2902	3039	3166	3249	3198	3103	2874	267	NS
DMI (g day^{-1})	131	156	155	150	155	147	133	9	**
NI (mg BW$^{0.67}$ kg^{-1})	625	1474	2102	2607	3363	4024	811	182	***
NEX (mg BW$^{0.67}$ kg^{-1})	261	575	789	1139	1647	2232	285	159	***
ND (mg BW$^{0.67}$ kg^{-1})	364	898	1312	1468	1716	1792	526	72	***

[1] N1=61, N2=124, N3=183, N4=239, N5=295, N6=364 g kg^{-1} dry matter and N7 is the counter-proof treatment (N1 + 4 g of L-lysine HCl (78 %) per kg of feed); [2] Mean value of two collection periods; [3] Residual standard deviation, expressed in the same units as the related variable; NS, not significant; **$p < 0.05$; ***$p < 0.001$.

Table 4 – Mean body weight (BW), dry matter intake (DMI), daily nitrogen intake (NI), daily nitrogen excretion (NEX) and daily nitrogen deposition (ND) obtained in nitrogen balance trials with females receiving graded levels of protein[1].

Diets	Females								
	Period I (6-21 days)[2]								
	N1	N2	N3	N4	N5	N6	N7	RSD[3]	p-value
BW (g)	254	312	342	373	383	363	270	122	NS
DMI (g day^{-1})	36	46	47	45	49	47	40	11	NS
NI (mg BW$^{0.67}$ kg^{-1})	895	2025	2876	3379	4461	5409	1210	80	***
NEX (mg BW$^{0.67}$ kg^{-1})	241	573	763	1092	1801	2591	360	83	***
ND (mg BW$^{0.67}$ kg^{-1})	654	1452	2113	2286	2660	2818	850	87	***
Diets	Period II (22-37 days)[2]								
	N1	N2	N3	N4	N5	N6	N7	RSD[3]	p-value
BW (g)	1192	1314	1398	1491	1381	1437	1235	210	NS
DMI (g day^{-1})	103	109	109	112	108	112	106	8	NS
NI (mg BW$^{0.67}$ kg^{-1})	903	1806	2573	3282	4129	5121	1150	155	***
NEX (mg BW$^{0.67}$ kg^{-1})	304	574	871	1261	1903	2661	347	113	***
ND (mg BW$^{0.67}$ kg^{-1})	599	1232	1703	2021	2225	2460	803	85	***
Diets	Period III (38-53 days)[2]								
	N1	N2	N3	N4	N5	N6	N7	RSD[3]	p-value
BW (g)	2270	2427	2589	2530	2530	2600	2342	238	NS
DMI (g day^{-1})	123	138	139	137	138	133	146	10	**
NI (mg BW$^{0.67}$ kg^{-1})	693	1517	2153	2819	3510	4080	1023	151	***
NEX (mg BW$^{0.67}$ kg^{-1})	292	554	897	1264	1890	2319	311	147	***
ND (mg BW$^{0.67}$ kg^{-1})	402	963	1255	1555	1620	1761	712	70	***

[1] N1=61, N2=124, N3=183, N4=239, N5=295, N6=364 g kg^{-1} dry matter and N7 is the counter-proof treatment (N1 + 4 g of L-lysine HCl (78 %) per kg of feed); [2] Mean value of two collection periods; [3] Residual standard deviation, expressed in the same units as the related variable; NS, not significant; **$p < 0.05$; ***$p < 0.001$.

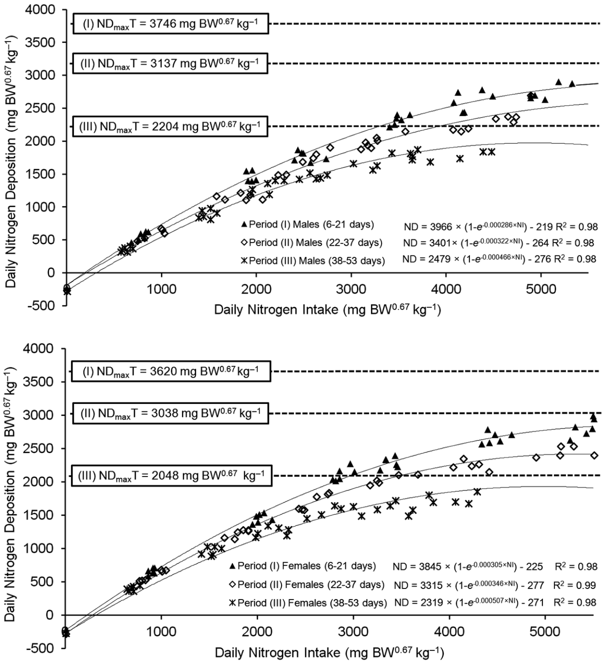

Figure 2 – Estimation of the theoretical potential for nitrogen deposition ($ND_{max}T$) in male and female broilers of the Cobb500 genotype based on the ratio between the daily nitrogen intake (NI) and the daily nitrogen balance (ND) at different ages.

Table 5 – Comparison between the nitrogen retention (NR) estimated in relation to the theoretical maximum nitrogen retention ($NR_{max}T$)[1] using the body weights (BW), feed intake (FI) and lysine requirements (Lys) provided by the management guide for Cobb500 broilers.

Period	BW	FI	Lys	Lys	NR[3]	NR
days	g	g day^{-1}	g kg^{-1}	mg $BW^{0.67}$ kg^{-1} day^{-1}		(%$NR_{max}T$)
			Cobb 2008			
			Males			
6 to 21	501	65	10.1	1090	2522	64
22 to 37	1633	168	9.5	1164	2405	71
38 to 53	3156	212	9.2	910	1860	75
			Females			
6 to 21	457	63	10.1	1070	2511	65
22 to 37	1442	153	9.5	1150	2417	73
38 to 53	2633	186	9.2	896	1791	77
			Cobb 2012			
			Males			
6 to 21	510	65	10.8	1143	2589	65
22 to 37	1583	173	9.6	1218	2461	72
38 to 53	3047	235	9.2	1028	1964	79
			Females			
6 to 21	488	61	10.8	1064	2506	65
22 to 37	1474	148	9.6	1093	2356	71
38 to 53	2795	198	9.2	920	1814	78

[1] $NR_{max}T$ = 3,966 and 3,845 (6 to 21 days); 3,401 and 3,315 (22 to 37 days); and 2,479 and 2,319 mg $BW^{0.67}$ kg^{-1} day^{-1} (38 to 53 days) for males and females, respectively; [2] bc^{-1}= 0.000058 and 0.000062 (6 to 21 days); 0.000066 and 0.000071 (22 to 37 days); and 0.000096 and 0.000104 (38 to 53 days) for males and females, respectively; [3] $NR = NR_{max}T - e^{(ln(NRmaxT)-(16 \times Lys \times bc^{-1}))}$.

riods I, II, and III, respectively. This increase in the *b* value was accompanied by a decline in the $ND_{max}T$ value that can be observed in Figure 2 and is characteristic of the modeling procedure. Consequently, the efficiency of lysine utilization, bc^{-1}, also increased. The mean values for the ratio between the *b* parameter and the lysine concentration, *c*, (from Table 2) were estimated at 58, 66, and 96×10^{-6} for males and 62, 71, and 104×10^{-6} for females during periods I, II, and III, respectively.

Results of the simulation using equation (8) and data from the Cobb500 management guides to estimate the NR under practical conditions are summarized in Table 5. The simulation showed that the potentials for nitrogen retention (NR) using the 2008 recommendations are 64 and 65 % of the $NR_{max}T$ (period I), 71 and 73 % of the $NR_{max}T$ (Period II), and 75 and 77 % of the $NR_{max}T$ (Period III) for males and females, respectively. For the 2012 management guide recommendations, the estimated potentials for the birds were 65 % of the $NR_{max}T$ for both sexes (period I), 72 and 71 % of the $NR_{max}T$ (period II), and 79 and 78 % of the $NR_{max}T$ (period III) for males and females, respectively. The difference in the potentials from 2008 to 2012 represented approximately 1 to 4 % for males but no more than 1 % for females.

Results for modeling of the Lys requirement data from equation (7) are summarized in Table 6 and demonstrate the application of the modeling procedure to establish Lys requirement data (mg day^{-1}) from the targeted growth potential or nitrogen retention (NR, mg $BW^{0.67}$ kg^{-1} day^{-1}) and bc^{-1}.

Based on the calculated values for bc^{-1}, the Lys intake required to achieve 65 % of the $NR_{max}T$ for both sexes (estimated from the simulation in Table 5) during period I was estimated to be 581 mg day^{-1} for males and 512 mg day^{-1} for females. During period II, the Lys intake to achieve 72 % of the $NR_{max}T$ for males and 71 % of the $NR_{max}T$ for females was estimated to be 1,538 mg day^{-1} for males and 1,340 mg day^{-1} for females. During period III, the Lys intake was estimated to be 2,171 mg day^{-1} for males and 1,674 mg day^{-1} for females to reach 79 and 78 % of the $NR_{max}T$, respectively.

Discussion

Dose response studies are generally based on the acceptance of a relationship between the content of the limiting amino acid in the diet and the growth response, but this ratio is only valid for the limiting position of the amino acid (Samadi and Liebert, 2006). By adding a small quantity of crystalline lysine in the N1 diet, it was possible to confirm that the amino acid tested in this study was in the limiting position because of the small incremental response in the birds being fed the N7 diet (Tables 3 and 4). This procedure was carried out to demonstrate that the response obtained by the relative deficiency of the test amino acid was independent of the

Table 6 – Calculations for the digestible lysine requirements (Lys) using equation (7) for males and females in each period for the targeted response estimated for the Cobb500 genotype using data from the manual for the strain (Cobb, 2012).

Period I (6-21 days)				
	Males		Females	
NR[1] (mg BW$^{0.67}$ kg^{-1} day^{-1})	2578		2499	
Efficiency (*bc^{-1}*)	0.000058		0.000062	
Lys (mg BW$^{0.67}$ kg^{-1} day^{-1})	1131		1058	
Lys (mg day^{-1})	581		512	
	Optimum level of dietary lysine (g kg^{-1})			
	Feed intake[2]	Lys	Feed intake[2]	Lys
	g day^{-1}	g kg^{-1}	g day^{-1}	g kg^{-1}
	55	10.6	55	9.3
	65	8.9	65	7.9
	75	7.7	75	6.8
Period II (22-37 days)				
	Males		Females	
NR (mg BW$^{0.67}$ kg^{-1} day^{-1})	2449		2354	
Efficiency (*bc^{-1}*)	0.000066		0.000071	
Lys (mg BW$^{0.67}$ kg^{1} day^{-1})	1205		1090	
Lys (mg day^{-1})	1538		1340	
	Optimum level of dietary lysine (g kg^{-1})			
	Feed intake[2]	Lys	Feed intake[2]	Lys
	g day^{-1}	g kg^{-1}	g day^{-1}	g kg^{-1}
	163	9.4	138	9.7
	173	8.9	148	9.1
	183	8.4	158	8.5
Period III (38-53 days)				
	Males		Females	
NR (mg BW$^{0.67}$ kg^{-1} day^{-1})	1958		1809	
Efficiency (*bc^{-1}*)	0.000096		0.000104	
Lys (mg BW$^{0.67}$ kg^{-1}day^{-1})	1016		910	
Lys (mg day^{-1})	2171		1674	
	Optimum level of dietary lysine (g kg^{-1})			
	Feed intake[2]	Lys	Feed intake[2]	Lys
	g day^{-1}	g kg^{-1}	g day^{-1}	g kg^{-1}
	225	9.6	188	8.9
	235	9.2	198	8.5
	245	8.9	208	8.1

[1] From Table 5: 65 % of the $NR_{max}T$ (3,966 and 3,845 mg BW$^{0.67}$ kg^{-1} day^{-1}for males and females, respectively) in period I for both sexes; 72 % of the $NR_{max}T$ for males (3,401 mg BW$^{0.67}$ kg^{-1} day^{-1}) and 71 % of the $NR_{max}T$ for females (3,315 mg BW$^{0.67}$ kg^{-1} day^{-1}) in period II; 79 % of the $NR_{max}T$ for males (2,479 mg BW$^{0.67}$ kg^{-1} day^{-1}) and 78 % of the $NR_{max}T$ for females (2,319 mg BW$^{0.67}$ kg^{-1} day^{-1}) in period III; [2] daily feed intake was based on the mean values for the given period in the management guide for the strain (Cobb, 2012) with extrapolation for 10 g above and 10 g below the mean.

level of dietary protein and was not influenced by the effects of dilution (Fisher and Morris, 1970).

The NMR value estimated in this study represents the amount of N that needs to be ingested to compensate for endogenous nitrogen losses, according to Samadi and Liebert (2006). The procedure used in this study is an alternative approach for estimating the maintenance requirements for nitrogen and amino acids because the values obtained from the traditional method, which uses the inevitable losses from the body protein in birds fed nitrogen-free diets, are underestimated (Samadi and Liebert, 2006). As an example of this, the total loss of endogenous nitrogen by growing broilers, estimated at 180 mg BW$^{0.75}$ kg^{-1} day^{-1} by Leeson and Summers (2001), is far below the values determined in this study when they are expressed in BW$^{0.75}$ (approximately 28 % for both males and females).

The procedure adopted here assumes that endogenous catabolism is largely or completely suppressed when the animal is receiving protein in the feed (Mitchel, 1924). The proportion of the maintenance requirement in relation to the total requirement increases with the age of the bird, and this element must be factored into the estimates. The NMR values obtained in this study are in accordance with the values estimated by Samadi and Liebert (2007) of 220, 260 and 273 mg BW$^{0.67}$ kg^{-1} day^{-1} for males and 223, 275 and 264 mg BW$^{0.67}$ kg^{-1} day^{-1} for females during periods I (10-25 days), II (30-45 days) and III (50-65 days), respectively.

As the maintenance requirements of the broilers become a larger proportion of the total requirement with increasing age, the nitrogen requirements to maximize protein deposition decrease. Due to differences among animals in their maintenance requirements and growth potentials, the response of the study population was curvilinear. In a curvilinear response, the utilization of a nutrient is characterized by (i) a linear phase at a suboptimal level, (ii) a curvilinear stage, and (iii) a decrease to the point at which the maximum response is obtained (Baker, 1986). In the curvilinear response obtained in this study (Figure 2), the $ND_{max}T$ represents the physiological limit of nitrogen deposition that is specific to the genotype.

The threshold values ($ND_{max}T$) observed decreased as age increased and were close to the results reported in the studies of Samadi and Liebert, (2007) for males (3,746 mg vs. 3,676 mg in age period I; 3,137 mg vs. 2,843 mg in age period II; 2,204 mg vs. 1,864 mg in age period III) and females (3,620 mg vs. 3,582 mg in age period I; 3,038 mg vs. 2,697 mg in age period II; 2,048 mg vs. 1,708 mg in age period III) fed diets that were first limiting in lysine.

The results from the simulation data (Table 5) indicate that there is still a capacity for retention that could be exploited by geneticists and nutritionists because the potentials for nitrogen retention currently represent only 65, 72, and 79 % (average value of 72 %) of the $NR_{max}T$ for males and 65, 71, and 78 % (average value of 71 %) of the $NR_{max}T$ for females during periods I, II, and III, respectively.

The differences in the potentials from 2008 to 2012 calculated from the simulation indicated a slight increase of approximately 1-4 % for males but no more than 1 % for females, a result of genetic improvements of this genotype over the last four years. Therefore, these differences can also be attributed to the fact that the growth curve describes the deposition under standard feeding conditions in which the physiological limit is not expressed.

The characterization of the growth patterns of this strain for protein deposition and the efficiency of utilization allows us to establish the appropriate levels for protein and amino acids to reach the desired potential tailored to the limiting conditions. Using the model parameters and the data obtained in this study, it is possible to establish an optimal lysine intake based on the targeted nitrogen retention (as a % age of the $NR_{max}T$) estimated from the actual Cobb500 recommendation (Table 6). The simulation indicated that to meet 65 % of the $NR_{max}T$ (period I), the concentration of digestible Lys in the diet should be 10.6 g kg^{-1} for males and 9.3 g kg^{-1} for females to obtain values close to the recommendations from the literature if a daily feed intake of 10 grams less than the recommended (Cobb, 2012) is considered. This recommendation is similar to that made by Goulart et al. (2008) of 10.6 g kg^{-1} (580 mg day^{-1}) for the initial 8-21 days phase for males.

Zaghari et al. (2002) reported that the digestible Lys requirement for maximum body weight gain from 6-21 days of age was 10.75 and 10.49 g kg^{-1} for males and females, respectively. The recommendation for females by these authors is higher than the recommendation in this study, but for males, the recommendations are very consistent. Han and Baker (1994) obtained a digestible lysine requirement estimate of 10.7 g kg^{-1} for males, and this recommendation is also similar. The values from these authors for maximum body weight gain can be compared to the estimated digestible lysine recommendation in this study due to the direct relationship between the Lys concentration in the diet and the increase in body weight and protein deposition (Mahdavi et al., 2012).

To meet 71 % of the $NR_{max}T$ for males and 72 % of the $NR_{max}T$ for females in period II, the concentration of digestible Lys in the diet should be 9.4 g kg^{-1} for males and 9.7 g kg^{-1} for females to obtain values close to the recommendations from the literature if a daily feed intake of 10 grams less than recommended (Cobb, 2012) for these periods is considered (Table 6). These values are close to the recommendations of Han and Baker (1994) of 9.9 g kg^{-1} for males and 9.1 g kg^{-1} for females to maximize weight gain during 3 to 6 wks of age. Similar recommendations for male broilers were made by Goulart et al. (2008) of 9.98 g kg^{-1} for the growth (22-42 d) phase to obtain the maximal performance. The estimated digestible lysine requirements in this study for periods I and II corresponded to approximately 93 % of the recommendations in the Brazilian Tables of 11.5 g kg^{-1} (10.7 g kg^{-1}) and 10.1 g kg^{-1} (9.4 g kg^{-1}) of digestible lysine at ages 8 to 21 days and 30 to 42 days for male broilers, respectively.

To meet 79 % of the $NR_{max}T$ for males and 78 % of the $NR_{max}T$ for females in period III, the concentration of digestible Lys in the diet should be 9.2 g kg^{-1} for males and 8.5 g kg^{-1} for females if the mean daily feed intake recommended for this period is considered (Table 6). Mahdavi et al. (2012) determined that the digestible Lys requirement for maximum body weight was 9.3 g kg^{-1} for both males and females for broilers between 35 and 49 days of age. Compared with the recommendation in this study, the estimate for males is very close, but that for females is higher. However, Mahdavi et al. (2012) concluded that high-yield broilers should be fed a minimum of 8.5 g kg^{-1} digestible Lys from 35 to 49 days of age, and this value is similar to the estimate made for female Cobb 500 broilers in this study.

The digestible lysine requirements estimated in this study are slightly below the calculations for 60 % of the theoretical potential for nitrogen retention (10-25 days: 11.0 g kg^{-1} lysine, 60 g daily feed intake; 30-45 days: 10.3 g kg^{-1} lysine, 140 g daily feed intake; 50-65 days: 9.6 g kg^{-1} lysine, 170 g daily feed intake) used in the study by Samadi and Liebert (2007) for the Cobb500 genotype. Urdanneta-Rincon et al. (2005) reported that both protein synthesis and breakdown increased when the levels of dietary Lys and crude protein were above those required for maximum growth. In this way, using the maximum potential for deposition as a reference for modeling the digestible lysine requirement allows for optimal digestible Lys levels to be supplied, resulting in a decrease in nitrogen excretion and a reduction in the feed costs, because protein is an expensive nutrient.

Conclusion

The model used to describe the maximum potential for N deposition in birds allows for estimates of the digestible lysine requirements in the diet. The flexibility of the model enables the requirements to be modeled while considering the potential for deposition and the feed intake of the birds within the range of practical performance data. The digestible lysine requirements were estimated at 10.6, 9.4, and 9.2 g kg^{-1} for males and 9.3, 9.7, and 8.5 g kg^{-1} for females during 6-21 days (period I), 22-37 days (period II), and 38-53 days (period III), respectively.

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