Maintenance valine, isoleucine, and tryptophan requirements for poultry

M. B. de Lima,*[,1] N. K. Sakomura,* J. C. P Dorigam,* E. P. da Silva,* N. T. Ferreira,* and J. B. K. Fernandes†

*Department of Animal Science, College of Agrarian and Veterinary Sciences, University Estadual Paulista, Jaboticabal, São Paulo, Brazil, 14884–900; and †Aquaculture Center of University Estadual Paulista 14884–900, Jaboticabal, São Paulo, Brazil

ABSTRACT Poultry maintenance requirements for valine, isoleucine, and tryptophan were measured by nitrogen balance using different unit systems. The nitrogen balance trial lasted 5 d with 48 h of fasting (with roosters receiving only water + sucrose) and the last 72 h for feeding and excreta collection. Forty grams of each diet first-limiting in valine, isoleucine, or tryptophan was fed by tube each day (3 d) to give a range of intakes from 0 to 101, 0 to 119, and 0 to 34 mg/kg BW d of valine, isoleucine, and tryptophan, respectively. A nitrogen-free diet containing energy, vitamins, and minerals, meeting the rooster requirements, was offered ad libitum during these three d. To confirm that the amino acids studied were limiting, a treatment was added with a control diet formulated by adding 0.24 g/kg of L-valine, 0.21 g/kg of L-isoleucine, and 0.10 g/kg of L-tryptophan to the diets with lower amino acid level. Excreta were collected during the last 3 d of the balance period and the nitrogen content of the excreta was analyzed. For each amino acid, a linear regression between nitrogen retention (NR) and amino acid intake was performed. The equations from linear regression were: NR = −98.6 (±10.1) + 2.4 (±0.2) × Val, NR = −46.9 (±7.1) + 2.3 (±0.1) × Ile, NR = −39.5 (±7.7) + 7.3 (±0.4) × Trp; where Val, Ile, and Trp are the intakes of valine, isoleucine, and tryptophan in mg/kg body weight per d, respectively. The valine, isoleucine, and tryptophan required to maintain the body at zero NR were calculated to be 41, 20, and 5 mg/kg body weight per d, respectively. For the system unit mg per kg of metabolic weight, the intake of valine, isoleucine, and tryptophan was 59, 32, and 9, respectively. Considering the degree of maturity of the animal and body protein content ($BP_m{}^{0.73} \times u$), the amounts of valine, isoleucine, and tryptophan required for maintenance were calculated to be 247, 134, and 37 mg per unit of maintenance protein ($BP_m{}^{0.73} \times u$) per d. Maintenance requirement is more adequately expressed as body protein content.

Key words: amino acids, breeder roosters, dilution technique, nitrogen retention, regression equation

2016 Poultry Science 95:842–850
http://dx.doi.org/10.3382/ps/pev380

INTRODUCTION

Determining the ideal amino acid intake in poultry husbandry, considering the specific requirements for maintenance and for egg production, has been suggested for more than seven decades (Heuser, 1941). Leveille and Fisher (1959) first tested and defined the maintenance concept as providing a minimal amount of amino acid to replace inevitable losses and maintain a constant nitrogen balance. Since then there has been little improvement in the methodologies to determine maintenance values although the concept of maintenance has been extensively studied (Fisher, 2013).

Several studies have established the maintenance requirements for the most limiting amino acids in practical diets for poultry, such as methionine + cysteine (Bonato et al., 2011), lysine (Bonato et al., 2011), and threonine (Nonis and Gous, 2008). For the amino acids valine, isoleucine, and tryptophan few studies have been conducted and the requirements obtained are outdated (Leveille and Fisher, 1959, 1960; Leveille et al., 1960; Ishibashi, 1973; Burnham and Gous, 1992). The studies published to date determining the amino acid requirements for maintenance in poultry have generated conflicting results (Leveille and Fisher, 1960; Ishibashi, 1973; Burnham and Gous, 1992, Baker et al., 1996). This divergence may reflect variations in the approaches these studies used, such as poultry of different physiological stages (growth and maturity) and different methodologies (nitrogen balance and comparative slaughter).

Most studies on the maintenance requirement express as mg of amino per kg of body weight or mg per kg of metabolic weight (Leveille and Fisher 1960; Baker et al., 1996; Edwards et al., 1997; Burnham and Gous, 1992; Sakomura and Coon, 2003). Nonetheless, because of the differences in body composition of birds especially

Received February 3, 2015.
Accepted October 29, 2015.
[1]Corresponding author: bernardino.michele@gmail.com

regarding the fat content, the ideal would be to express amino acid maintenance requirement on the basis of protein weight of the bird, which would allow comparisons between studies (Burnham and Gous, 1992; Martin et al., 1994; Nonis and Gous, 2008; Bonato et al., 2011). Thus, there is still a need to investigate how the poultry respond to the intake of the amino acids valine, isoleucine, and tryptophan in maintenance, to generate values that adequately represent the requirements of these amino acids in this state.

Therefore, the aim of this study was to determine the specific requirements of digestible valine, isoleucine, and tryptophan for maintenance by different unit systems using Cobb 500 breeder roosters as an animal model. Knowledge about the specific requirements of these amino acids at maintenance will contribute to future studies aiming to improve the productivity of the poultry industry.

MATERIALS AND METHODS

The trials were conducted at the Poultry Science Laboratory of the São Paulo State University, UNESP Jaboticabal, São Paulo, Brazil. The Animal Ethics and Welfare Committee of UNESP (CEUA) approved all experimental procedures used in the research under the Protocol number 9999/14.

Birds and Housing

Three nitrogen balance studies were performed and 144 Cobb 500 breeder roosters were housed individually. The roosters were housed in metabolic cages with individual feeder and nipple-type drinkers. During the experimental period, light was provided 24 h per d.

Valine Trial

In the valine trial 42 roosters were used, distributed into six treatments and six replicates, with an average weight of 4.15 ± 0.55 kg. To confirm that the amino acid studied was indeed limiting and that responses were due to the limitation of the test amino acid and not protein, we performed one more treatment consisting of a control diet. A summit diet was formulated containing 11.75 g/kg of digestible valine and a nitrogen-free diet was formulated based on corn starch and rice husk considering the same nutritional levels as in the summit diet, except for protein and amino acids (Table 1). The experimental levels of the amino acids were obtained from the dilution technique (Fisher and Morris, 1970) and had the following proportions in summit and nitrogen-free diets: 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0. The valine levels ranged from 0 to 1.175% in the diet. A total of 0.24 g/kg of L-valine was added in the diets with lower amino acid level (V2) up to the second amino acid level in the diet (V3).

Isoleucine Trial

In the isoleucine trial, 54 roosters were used, distributed into eight treatments and six replicates each, with an average weight of 5.69 ± 0.48 kg, and one additional treatment was added (control diet), in the same way as described the valine trial. A summit diet was formulated containing 18.18 g/kg of digestible isoleucine and a nitrogen-free diet was also formulated (Table 1). The isoleucine levels ranged from 0 and 1.818% of the diet. The experimental levels were obtained with the dilution technique. For the control diet 0.21 g/kg of L-isoleucine was added to the diets with lower amino acid level (I2) up to the second amino acid level in the diet (I3).

Tryptophan Trial

In the tryptophan trial 48 birds were used, distributed into seven treatments and six replicates each with an average weight of 6.11 ± 0.38 kg. In this study, a control diet was also added. A summit diet was formulated containing 5.70 g/kg of digestible tryptophan and a nitrogen-free diet was also formulated (Table 1). The experimental levels of the amino acids were obtained from the dilution technique (Fisher and Morris, 1970) and had the following proportions in summit and nitrogen-free diets: 0:100, 22:78, 33:67, 44:56, 56:44, 67:33, 78:22, and 100:0. The tryptophan levels ranged from 0 to 0.570% of the diet. For the control diet 0.10 g/kg of L-tryptophan was added to the diets with lower amino acid level (T2) up to the second amino acid level in the diet (T3).

Experimental Diets

Based on the recommendations of Rostagno et al. (2011) the requirement for the amino acid tested was multiplied by 0.4 and 0.8 for other amino acids, creating a minimum relative deficiency of 40% between the test amino acid and other amino acids, as suggested by Siqueira et al. (2011) and Bonato et al. (2013). Based on the ideal profile of amino acid/lysine, summit diets with high protein content were formulated (Table 1), meeting the requirements for energy, minerals, and other nutrients proposed by Rostagno et al. (2011). In all diets, the minimum contents of potassium, calcium, available phosphorus, and sodium were 7.50, 6.50, 3.00, and 2.30 g/kg, respectively.

Experimental Procedure and Data Collection

The experiment lasted 120 h and in the first 48 h the birds went through a period of fasting to empty the gastrointestinal tract. In this period, they received

Table 1. Composition (g/kg) of the summit and N-free diet.

Ingredients	Summit valine	N-free valine	Summit isoleucine	Summit tryptophan	N-Free
Corn starch		431.9			673.3
Soybean meal (460 g/kg)	224.9		342.6	364.1	
Corn	675.9		370.0	275.6	
Corn gluten meal (600 g/kg)			150.2	202.9	
Sugar		350.0			150.0
Rice husk		100.0			108.9
Soybean oil	21.5	30.0	34.4	32.2	24.7
Dicalcium phosphate	11.4	16.2	10.1	10.0	16.2
Potassium chloride	2.7	14.3			14.3
Sodium chloride	5.3	5.5	5.4	5.4	5.5
Limestone	7.9	4.3	8.1	8.0	4.1
Premix[1]	3.0	3.0	3.0	3.0	3.0
L-Arginine (980 g/kg)	6.3		12.4	15.9	
L-Valine (970 g/kg)	5.2		13.0	15.9	
DL-Methionine (980 g/kg)	7.3		12.1	14.5	
L-Lysine HCl (780 g/kg)	5.2		10.8	14.0	
L-Isoleucine (985 g/kg)	6.2		7.1	12.5	
L- Threonine (980 g/kg)	5.6		9.7	11.9	
L- Phenylalanine (990 g/kg)	5.0		4.5	5.9	
L-Glycine (985 g/kg)	1.0		3.6	5.3	
L-Leucine (980 g/kg)	4.1				
L-Tryptophan (980 g/kg)	1.6		3.1	2.9	
Inert (Sand)		44.8			
Total	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated nutritional composition and amino acids (g/kg)[2]					
Metabolizable energy (MJ/kg)	13.40	13.40	13.40	13.40	13.40
Crude protein[3]	196/227	5.69/5.94	352/374	407/394	0.14/0.95
Lysine	11.14		19.49	22.87	
Methionine + cystine	11.70		20.50	24.05	
Methionine	9.51		16.35	19.42	
Tryptophan	3.23		5.67	5.70	
Threonine	10.76		18.86	22.13	
Arginine	15.63		27.34	32.08	
Valine	11.75		24.70	28.98	
Phenylalanine	12.14		19.04	22.49	
Phenylalanine + tyrosine	17.25		30.20	35.43	
Glycine + Serine	13.92		24.36	28.58	
Histidine	3.94		6.55	7.19	
Isoleucine	12.11		18.18	24.89	
Leucine	17.25		30.20	35.43	

[1]Premix provided per mg/kg diet: retinyl acetate, 3.60; cholecalciferol, 0.055; all-rac-α-tocopherol acetate, 30; thiamine, 2.2; riboflavin, 6; pyridoxine.HCl, 3.3; cobalamin, 0.016; nicotinamide, 53; pantothenic acid, 13; menadione, 2.5; folic acid, 1; Se (as Na_2SeO_3), 0.25; Mn (as $MnSO_4.H_2O$), 75; Fe(as $FeSO_4.H_2O$), 50; Zn (as ZnO), 70; Cu (as $CuSO_4.5H_2O$), 6.5; I (as KIO_3), 1.5.
[2]Digestible amino acid.
[3]Calculated and Analyzed crude protein (N ∗ 6.25), respectively.

60 mL of water with sucrose (50% each) once a day. For the next 72 h, the birds were fed 40 grams of the experimental diets daily. At the same time, nitrogen-free diet was provided ad libitum to maintain energy homeostasis and minimize metabolic and endogenous losses of birds. As a marker for excreta, 1% of iron oxide was used at the beginning and end of the collection period. Trays covered with plastic were placed under the metabolic cages to collect the excreta. The excreta were collected twice per d (at 8 h and 16 h) and placed in plastic containers that were kept at −20°C. Feed intake was measured for the 72 h of the feeding phase, determined by feed consumption provided by forced feeding plus the nitrogen-free diet provided ad libitum in the feeder. At the end of each trial, two birds were killed and feathers were separated from the carcass for further analysis.

Analytical Procedures

The amount of excreta from each animal was weighed after the 3 d of collection. The excreta were homogenized in a blender, and samples were weighed into Petri dishes. After, the samples were lyophilized for 72 h. Next, samples were ground in a ball mill for 2 minutes. These dried samples of the experimental diets, excreta, and carcass (free of feathers) were analyzed for crude protein by Kjeldahl (method 2001.11) and ether extract (method 920.39) for carcass (free of feathers), according to AOAC (1995) procedures.

Statistical Analysis

The nitrogen retention (NR) was determined by the difference between nitrogen intake and excretion. The

Table 2. Average values for feed intake (FI), valine intake (ValI), and nitrogen retention (NR) in different systems of units.

Diet	Levels (%) Valine	FI g/d	ValI mg/kg BW d	ValI mg/kg $BW^{0.75}$ d	ValI mg/kg $BP_m^{0.73}$ d	NR mg/kg BW d	NR mg/kg $BW^{0.75}$ d	NR mg/kg $BP_m^{0.73}$ d
V1	0.000	111.0	0.0	0.0	0.0	−96.3	−137.5	−577.0
V2	0.235	124.5	21.8	30.5	129.8	−37.8	−53.0	−222.5
V3	0.470	108.6	43.4	61.2	256.8	−10.4	−15.8	−66.0
V4	0.705	110.8	60.0	86.2	362.4	55.6	79.0	332.0
V5	0.940	126.3	81.8	116.0	488.3	102.8	145.5	611.0
V6	1.175	125.8	100.8	143.2	601.6	141.6	199.8	839.0
	Root MSE	16.1	18.3	13.9	42.6	40.1	54.9	230.4

V = valine diets; BW = Body weight; $BP_m^{0.73}$ = Body protein at maturity; Root MSE = root mean square error; Val = Valine.

Table 3. Average values for feed intake (FI), isoleucine intake (IleI), and nitrogen retention (NR) in different systems of units.

Diet	Levels (%) Isoleucine	FI g/d	IleI mg/kg BW d	IleI mg/kg $BW^{0.75}$ d	IleI mg/kg $BP_m^{0.73}$ d	NR mg/kg BW d	NR mg/kg $BW^{0.75}$ d	NR mg/kg $BP_m^{0.73}$ d
I1	0.000	112.3	0.0	0.0	0.0	−58.2	−89.7	−379.5
I2	0.404	142.8	27.0	41.5	175.0	20.5	31.2	131.3
I3	0.606	148.6	39.6	60.6	256.6	67.2	103.6	438.0
I4	0.808	133.5	50.8	79.17	335.8	68.8	106.7	451.5
I4	1.010	153.8	64.7	100.5	424.7	94.2	145.5	615.8
I6	1.212	144.8	78.2	120.6	510.6	140.6	216.8	916.6
I7	1.414	155.5	94.5	144.0	608.3	189.0	286.8	1211.5
I8	1.818	156.2	118.6	183.2	774.4	221.4	342.0	1448.2
	Root MSE	29.86	6.04	6.96	28.74	27.39	40.54	30.83

I = Isoleucine diet; BW = Body weight; $BP_m^{0.73}$ = Body protein at maturity; Root MSE = root mean square error.

nitrogen retention responses to the amino acid intakes were expressed in three unit systems: 1) mg per kg of body weight (BW), mg/BW per d; 2) mg per kg of metabolic weight ($BW^{0.75}$), mg/$BW^{0.75}$ per d; and 3) mg per unit of metabolic protein weight at maturity ($BP_m^{0.73} \times u$), mg/ $BP_m^{0.73} \times u$ per d, where u is the maturity rate, given by the relation between protein weight at time (BP_t) and protein weight at maturity (BP_m) where $u = BP_t/BP_m$), according to Burnham and Gous (1992). In these trials, u was considered equal to 1, because the birds were adults and had reached maturity. The metabolic protein weight at maturity was obtained by multiplying the percentage of protein and the weight of carcass free of feathers, raised to power 0.73. A linear regression between amino acid intake and NR was carried out. The requirement for maintenance was considered as the amount of amino acid to maintain NR equal to zero (NR = 0), as described by Sakomura and Rostagno (2007). Statistical analyses were performed using the PROC REG from SAS 9.2 software (SAS Institute, 2010).

RESULTS

The assumptions of normality of errors and homogeneity of variance were tested and satisfied. The responses of the roosters to the control diets showed that the amino acids studied were limiting in the diet, because they provided retentions between treatments V2, I2, T2 and V3, I3, T3, validating this study.

Valine Trial

The results of feed intake, amino acid intake, and nitrogen retention for different systems of units in the valine trial are presented in Table 2. The feed intake observed in the valine trial was 118 ± 16 g/d. The experimental diets provided ranges from negative to positive NR values. As the amino acid intake increased, the NR values also increased. In the valine trial, three levels showed negative NR.

Isoleucine Trial

The results of feed intake, amino acid intake, and nitrogen retention for the different systems of units in the isoleucine trial are shown in Table 3. The feed intake observed in the isoleucine trial was 143 ± 29.86 g/d. In the isoleucine trial, there were only negative responses on NR values in the nitrogen-free diet.

Tryptophan Trial

The results of feed intake, amino acid intake, and nitrogen retention for the different systems of units in the tryptophan trial are shown in Table 4. The feed intake observed in the tryptophan trial was 167 ± 29.86 g/d.

Table 4. Average values for feed intake (FI), tryptophan intake (TrpI), and nitrogen retention (NR) in different systems of units.

Diet	Levels (%) Tryptophan	FI g/d	TrpI, mg/kg BW d	TrpI, mg/kg $BW^{0.75}$ d	TrpI, mg/kg $BP_m^{0.73}$ d	NR mg/kg BW d	NR mg/kg $BW^{0.75}$ d	NR mg/kg $BP_m^{0.73}$ d
T1	0.000	158.0	0.0	0.0	0.0	−48.8	−76.2	−322.0
T2	0.095	154.0	6.0	9.8	40.5	13.8	21.3	89.5
T3	0.190	162.8	12.0	18.8	79.8	61.2	95.7	405.5
T4	0.285	162.7	17.5	27.7	118.0	82.8	129.8	550.0
T5	0.380	166.2	23.2	36.8	155.8	121.2	191.2	810.2
T6	0.475	172.3	29.7	46.3	196.5	182.8	287.0	1215.2
T7	0.570	194.7	34.0	53.7	227.2	207.0	327.5	1388.3
	Root MSE	33.5	1.5	1.7	7.0	29.2	44.4	187.7

T = Tryptophan diets BW = Body weight; $BP_m^{0.73}$ = Body protein at maturity; Root MSE = root mean square error.

Table 5. Maintenance requirements for digestible valine, isoleucine, and tryptophan estimated from linear regression equation based on three units.

Amino acid	Regression equation[1]	R^2	Requirement
Valine			
mg/kg BW d	NR = −98.6 (± 10.1) + 2.4 (± 0.2) × Val	0.90	41
mg/kg $BW^{0.75}$ d	NR = −140.6 (± 14.7) + 2.4 (± 0.2) × Val	0.90	59
mg/kg $BP_m^{0.73}$ d	NR = −591.7 (± 62.2) + 2.4 (± 0.2) × Val	0.90	247
Isoleucine			
mg/kg BW d	NR = −46.9 (± 7.1) + 2.3 (± 0.1) × Ile	0.92	20
mg/kg $BW^{0.75}$ d	NR = −72.6 (± 11.1) + 2.3 (± 0.1) × Ile	0.92	32
mg/kg $BP_m^{0.73}$ d	NR = −307.9 (± 46.7) + 2.3 (± 0.1) × Ile	0.92	134
Tryptophan			
mg/kg BW d	NR = −39.5 (± 7.7) + 7.3 (± 0.4) × Trp	0.92	5
mg/kg $BW^{0.75}$ d	NR = −62.9 (± 12.5) + 7.3 (± 0.4) × Trp	0.92	9
mg/kg $BP_m^{0.73}$ d	NR = −266.7 (± 52.4) + 7.3 (± 0.4) × Trp	0.92	37

[1]The data of the control diet were not utilized in the statistical analyses. BW = Body weight; BPm = Body protein at maturity; NR = nitrogen retention; Val = valine; Ile = isoleucine; Trp = tryptophan.

In the tryptophan trial, only negative responses were observed in regard to NR values in the nitrogen-free diet.

The regression equations obtained for all the different systems of units regarding the valine, isoleucine, and tryptophan trials are shown in Table 5. The coefficients of determination (R^2) in the studies were 90%, 92%, and 92% for valine, isoleucine, and tryptophan trials, respectively, indicating that there was a good fit of the data.

To cancel the unit effect, the observed response (Y) and amino acid intake (X) studied were calculated as a percentage of the maximum value observed in X and Y axes for each amino acid and plotted in a standardized scale of 0 to 100%. The data are shown in Figures 1, 2, and 3 for the valine, isoleucine, and tryptophan trials, respectively. The different unit systems showed overlapping results and similar emerging slope for each of the amino acid trials tested. The systems mg/kg $BW^{0.75}$ per d and mg/kg $BP_m^{0.73}$ per d were remarkably similar in the case of the valine and tryptophan trials.

DISCUSSION

The specific maintenance requirements for valine, isoleucine, and tryptophan for poultry were estimated with different unit systems in order to search for values that represent the actual need for these amino acids in this physiologic state.

The maintenance requirements determined for valine in the present study (41 mg/kg BW d) differed from those reported in other studies, which were 55 mg/kg BW d (Leveille and Fisher, 1960), 15 mg/kg BW d (Ishibashi, 1973), and 60 mg/kg BW d (Fisher, 1983). The lower value reported by Ishibashi (1973) can be explained by the sigmoidal function used by this author because, as explained by Baker (2005), any mathematical function affects the interpretation of the results that, in turn, can influence the estimated value. The difference between the values found herein and the studies by Leveille and Fisher (1960) and Fisher (1983) may be due to the feeding adjustments that were performed to improve the energy homeostasis of these birds.

The amino acids necessary to maintain the integrity of body proteins in birds include those that are inevitably lost through the digestive tract, peeling, and feather loss (Baker, 2005; Sakomura and Rostagno 2007; Kebreab et al., 2008; Wu, 2014). Some researchers argue that the maintenance requirements are positively correlated with the amino acid composition of feathers (Leveille et al., 1960; Silva et al., 2014). Valine is found in high concentration in feather's protein (69.2 mg/g) (Leveille et al., 1960), therefore, it is possible that birds

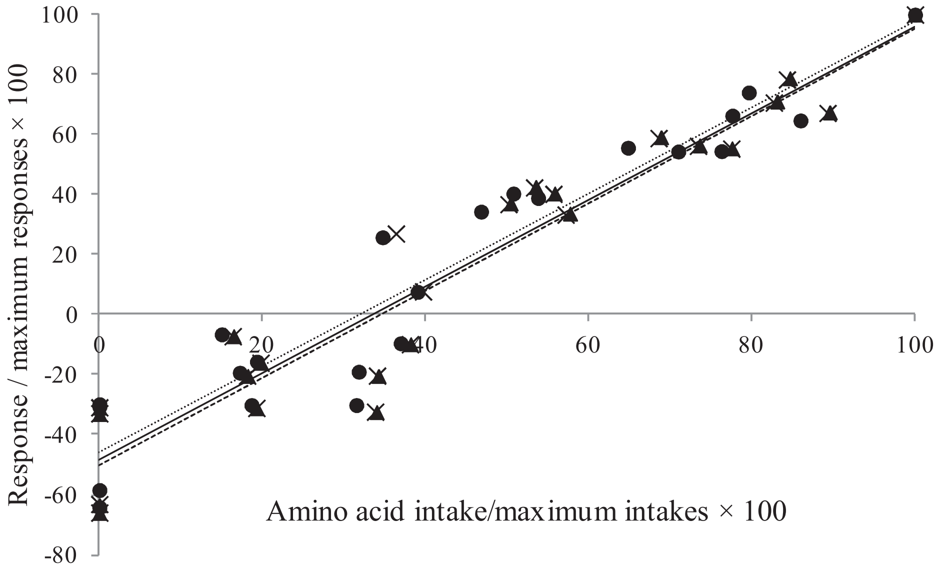

Figure 1. Graph representing a linear regression between nitrogen retention and valine intake, expressed in relation to a maximum response (%). (......, •) mg/kg BW per d; (—, ×) mg/kg $BW^{0.75}$ per d; (- -, ▲) mg/$BPm^{0.73}$ per d.

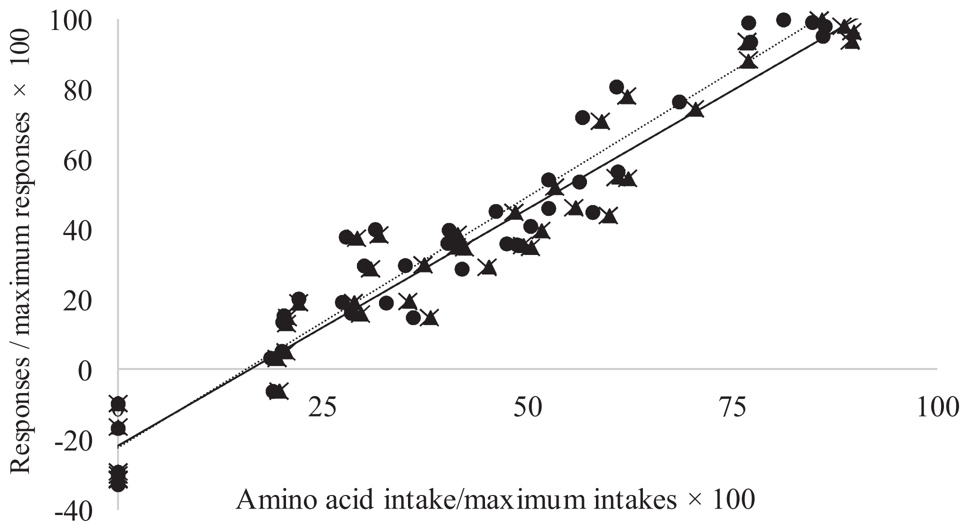

Figure 2. Graph representing a linear regression between nitrogen retention and isoleucine intake, expressed in relation to a maximum response (%). (......, •) mg/kg BW per d; (—, ×) mg/kg $BW^{0.75}$ per d; (- -, ▲) mg/$BPm^{0.73}$ per d.

have an increased requirement for valine for the keratin synthesis in order to replace feather losses.

The isoleucine requirement for maintenance of the breeder roosters was calculated to be 20 mg/kg BW d. The daily values published in the literature vary from 15 to 60 mg/kg BW d (Burnham and Gous, 1992; Leveille and Fisher, 1960; Huyghebaert et al., 1991; Ishibashi, 1973). However, the diet and strain of birds used in this study differed from the studies of these authors, which may account for the differences in the results. The effect of strain can be found in the intercept of the equation, because the linear coefficient of the adjusted models NR = −46.9 + 2.3 × Ile versus NR = −135 + 2.21 × Ile (Burnham and Gous, 1992) were close, i.e., the strains have similar responses. However, the intercept of the equations cannot be explained in a simple way. According to Kebreab et al. (2008), there are several factors such as the dietary energy: protein ratio that can affect the metabolic and endogenous losses and consequently the value of the maintenance requirement. The diets employed in this study had higher energy: protein ratio, higher concentration of isoleucine and dietary protein, and lower leucine in dietary protein than those described in Burnham and Gous (1992). High energy: protein ratio and the inclusion of lipid sources in the diet possibly reduce the oxidation of leucine and isoleucine (Adibi, 1976). Moreover, addition of fatty acids reduces the decarboxylation of branched chain amino acids and this may be related to the nitrogen-sparing effect described by some authors (Adibi, 1976), which consequently reduces endogenous losses. The isoleucine: leucine ratio in our diet was 1:1.4, while Burnham and Gous (1992) used 1: 2.8. Higher concentration of isoleucine in relation to

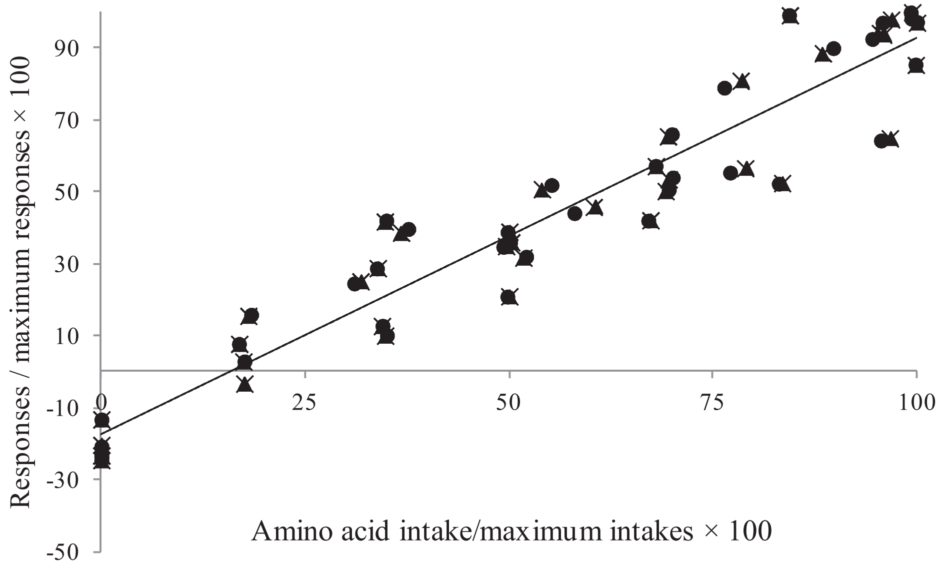

Figure 3. Graph representing a linear regression between nitrogen retention and tryptophan intake, expressed in relation to a maximum response (%). (......, •) mg/kg BW per d; (—, ×) mg/kg $BW^{0.75}$ per d; (–, ▲) mg/kg $BPm^{0.73}$ per d.

leucine can improve the use of isoleucine. Indeed, the increase in dietary leucine has been reported as a factor that reduces the efficiency of utilization of isoleucine (Allen and Baker, 1972). This may have contributed to the reduced endogenous and metabolic losses, resulting in the value of −46.9 mg/kg BW per d in this study and −135 mg/kg BW per d found by Burnham and Gous (1992). Thus, the lower values of isoleucine requirement found may be due to reduced oxidation of this amino acid and reduced endogenous and metabolic losses caused by the inclusion of lipid sources and the ratio of isoleucine: leucine in the diet.

The maintenance requirement for tryptophan determined in this study was 5 mg/kg BW d and was similar to the values obtained by Ishibashi (1973) and Leveille and Fisher (1960) of 5 and 7 mg/BW for roosters, respectively. Our results were also close to the values obtained in studies on different species using different methodologies published by Baker et al. (1966), Wethli and Morris (1978), and Kim et al. (1997), who determined values of 5, 6, and 5 mg/kg BW d for swine, laying hens, and broilers, respectively. For pullets, the values obtained were 10 mg/kg BW d (Morris and Wethli, 1978) and 11 mg/kg BW d (McDonald and Morris, 1985). The value obtained for laying hens was 6 mg/kg BW d (Wethli and Morris, 1978).

Many researchers recommend requirements for maintenance using the system mg/kg BW per d or mg/kg $BW^{0.75}$ per d (Leveille and Fisher, 1960; Leveille et al., 1960). However, these systems do not consider the differences in body composition. According to Taylor (1980) and Emmans (1989), the requirement for maintenance should be related to protein weight of the bird and maturity of body protein (u), which is the ratio of the weight of protein of the present state divided by the weight of the mature protein. The scale used by these authors does not consider the fat content, which may eliminate the imprecision in determining the amino acid requirements due to variation of the fat content, which vary widely in birds of the same genotype and body weight (Gous et al., 1984; Burnham and Gous, 1992; Nonis and Gous, 2008; Siqueira et al., 2011; Bonato et al., 2011; Bonato et al., 2013).

The effect of the unit systems in defining valine, isoleucine, and tryptophan maintenance requirements was also evaluated. The values were calculated as a percentage of the maximum observed value in the X and Y axes and plotted on a standardized scale from 0 to 100% (Figures 1, 2, and 3). The lines representing the different unit systems showed great overlapping, especially for the systems mg/kg $BW^{0.75}$ per d and mg/kg ${BP_m}^{0.73}$ per d. This similarity probably reflects the fact that the same samples were used to perform all calculations.

The system mg/kg ${BP_m}^{0.73} \times u$ per d allows the proportion of the maintenance requirement for any age to be extrapolated, as long as the degree of maturity of the protein is considered. To illustrate this, the data from Bae et al. (1999) for valine and isoleucine and from Kim et al. (1997) for tryptophan were used. These studies determined requirements of valine, isoleucine, and tryptophan for maintenance in growing birds (average weight of 0.269 kg). Based on their maintenance requirements, the valine, isoleucine, and tryptophan intakes for maintenance were calculated to be 1.1, 1.0, and 0.6 mg/bird, respectively.

Considering again the data from Bae et al. (1999) and Kim et al. (1997), we calculated body weight, metabolic weight, and maturity of body protein and applied the maintenance requirements found in this study for valine, isoleucine, and tryptophan. For valine the intakes calculated with systems mg/kg BW d, mg/$BW^{0.75}$, and mg/kg ${BP_m}^{0.73}$ d × u were 11.2, 22.3, and 11.6 mg/bird, respectively. For isoleucine the values found for the systems mg/kg BW d, mg/kg $BW^{0.75}$ d, and mg/kg ${BP_m}^{0.73}$ d × u, were 5.7, 12.5, and 6.4 mg/bird, respectively. For tryptophan the intakes calculated using the systems mg/kg BW d, mg/kg $BW^{0.75}$ d, and mg/kg ${BP_m}^{0.73}$ d × u, were 1.2, 3.2, and 1.4 mg/bird, respectively. Based on these comparisons, it is clear to see an underestimation of amino acid intake for maintenance when the maintenance requirements recommended by Bae et al. (1999) and Kim et al. (1997) are used. The values found in the present study can be attributed to the methodological characteristics adopted including the use of breeder roosters, the feeding system, and the mathematical function employed.

Burnham and Gous (1992) suggested that the best way to express the amino acid requirement for maintenance in growing and adult birds is according to protein weight, because there is no demand for amino acid to maintain the adipose tissue. In addition, the body lipid content is influenced by environmental factors and diet (Emmans, 1989; Gous et al., 1999), mainly after maturity. However, the protein weight of the animal remains constant (Gous et al., 1999; Marcato et al., 2008; Silva et al., 2013) and the increase in weight occurs due to increase in body fat (Gous et al., 1999; Marcato et al., 2008). Thus, the maintenance unit based on protein weight and degree of maturity is the best way to express the requirements. Based on this unit, the determined requirements are: 24, 134, and 37 mg/kg ${BP_m}^{0.73}$ per d for valine, isoleucine, and tryptophan, respectively.

This study shows that determination of amino acid requirements at maintenance can vary according to the type of animal and methodology used. It is believed that the feeding and the calculations based on protein weight and degree of maturity employed herein express more precisely the amino acid requirements at maintenance for poultry than do other systems. The data concerning calculated requirements for the amino acids valine, isoleucine, and tryptophan may contribute to future studies aiming to improve poultry production, through use in factorial models to increase precision in diet formulation.

ACKNOWLEDGMENTS

We thank the São Paulo Research Foundation (FAPESP) for financial support and scholarship provided to the first author (grant nº 2013/13957-1).

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