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The response of broilers during three periods of growth to dietary valine

Nayara Tavares Ferreira [a,*], Ricardo de Albuquerque [b], Nilva Kazue Sakomura [a], Juliano César de Paula Dorigam [a], Edney Pereira da Silva [a], Maria Fernanda C. Burbarelli [b], João Guilherme Ferreira [b], Robert Mervyn Gous [c]

[a] Department of Animal Sciences, Faculdade de Ciências Agrárias e Veterinárias-UNESP, Via de Acesso Paulo Donato Castelllane, 14884900, Jaboticabal, São Paulo, Brazil
[b] Department of Animal Production and Nutrition, Faculdade de Medicina Veterinária e Zootecnia-USP, Av. Duque de Caxias Norte, Pirassununga, São Paulo, Brazil
[c] University of KwaZulu-Natal, Pietermaritzburg, South Africa

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A B S T R A C T

In diets based on corn and soybean meal valine is considered the fourth limiting amino acid. Despite that, there have been few studies to date on the response of broilers to dietary valine and most of them use empirical procedures leading to a great variation in the recommendations. Thus, the aim of this study was to quantify the performance and body composition of broiler chickens subjected to different intakes of digestible valine using the dilution technique. Three trials were conducted separately with Cobb 500 broilers in the starter (1–14 days), grower (14–28 days) and finisher phase (28–42 days). In the starter and grower phases, the birds were distributed in a completely randomized design with eight treatments (seven levels of valine and a control treatment), with seven replicates, each consisting of 12 birds for starter and grower phases while 10 birds were used in the finisher phase. Basal diets were formulated by dilution technique, being one summit diet with valine as the first limiting amino acid and diluted with a nitrogen-free diet to obtain the intermediary levels. Valine intake for maximum weight gain, valine deposition, and protein deposition in the defeathered body and feather were estimated with broken-line models. Body fat content was calculated with linear regressions. Results indicate that to maximize performance of broiler chickens across several parameters, the recommended requirement of digestible valine for the starter, grower and finisher diet is 226, 637 and 1231 mg/bird/day, respectively. These recommendations allow achieving optimum performance in the broiler production system but they can be different depending on way birds are sold (whole chicken or cut-up) and age of the birds at slaughter.

1. Introduction

Several essential amino acids are currently available as dietary supplements in the poultry industry, particularly those that are most limiting in the feed. The availability of synthetic amino acids enables the amino acid requirements for growing birds

* Corresponding author. Fax: +55 1632097454.
E-mail address: nayaratf@hotmail.com (N.T. Ferreira).

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to be more precisely met, as a result, has improvements in the poultry nutrition. Specifically, it has enabled the formulation of feeds based on digestible amino acids. Moreover, the use of the concept of ideal protein for diet formulation (Baker and Han, 1994) allowed the reduction of the amount of protein in the diet, hence cost, therefore reducing the excretion of nitrogen to the environment.

In order to further reduce the levels of crude protein in broiler feeds a more precise estimation of the response to the main limiting essential amino acids is needed. These levels may be estimated using a factorial approach, accounting for the amount required for maintenance and protein deposition of body throughout the growing period, using as tool, for example the EFG Software (2014) or by conducting response trials in which each amino acid of interest is made to be first-limiting in the series of feeds applied (Gous, 1980; Gous and Morris, 1985). Responses to the main limiting amino acids, that being methionine, lysine and threonine which are routinely supplemented in the diets of broiler chickens are now well established. As the amino acid composition of the protein differs between feed ingredients, the next-limiting amino acid that being valine, isoleucine, tryptophan or arginine, will depend on the specific composition of the broiler feed (Corrent and Bartelt, 2011).

In diets based on corn and soybean meal, valine is the fourth limiting amino acid (Kidd and Hackenhaar, 2006; Corzo et al., 2009). Thornton et al. (2006) suggested that valine is the next limiting amino acid after threonine in diets in which ingredients of animal origin are excluded, especially in the grower and finisher periods, when a higher proportion of grains is used (Corzo et al., 2004).

However, there have been few studies to date on the response of broilers to dietary valine. Suggested inclusion rates in broiler feeds range from 7.4 to 9.0 g/kg diet (Farran and Thomas, 1990; Bae et al., 1999; Mack et al., 1999; Baker et al., 2002; Corzo et al., 2004,2009; Tavernari et al., 2013) but these estimates can show considerable variation depending on the mathematical procedures used to estimate them and the response parameters measured. Moreover, previous studies used supplementation techniques to determine valine requirements. This technique has been criticized because of the successive increase of the limiting amino acid in the basal diet induces an unbalance of other amino acids. An alternative to this technique is the dilution technique proposed by Fisher and Morris (1970) which consists of sequentially diluting a high protein diet with an iso-energetic diet free of protein to obtain intermediate levels of the amino acid evaluated, which ensures that the ratio between the amino acids remains constant (Gous, 1980).

Therefore, the aim of this study was to quantify the performance and body composition of broiler chickens subjected to different intakes of digestible valine using the dilution technique.

2. Material and methods

The experiment was conducted at the Faculty of Veterinary Medicine and Animal Science, University of São Paulo, Campus Pirassununga. This experiment was authorized by the Ethics Committee on animal use of the University of São Paulo (protocol n° 2153/2011).

2.1. Birds, experimental design and diets

Three experiments were conducted to measure the response of broilers to digestible valine in the starter (1–14 days), grower (14–28 days) and finisher (28–42 days) phases. The birds were weighed individually at day old, at 14 and 28 d in order to standardize body weight of the experimental units. The seven levels of dietary digestible valine and the control diet were distributed in a completely randomized design, using seven replicates of 12 birds for starter and grower phases while 10 birds were used in the finisher phase. In the experiments, male broilers of the Cobb 500 strain were used, with initial body weights of 49.2 ± 0.11 g at 1 day, 278 ± 0.87 g at 14 days and 1099 ± 3.56 g at 28 day of age.

The birds which were used to grower and finisher phase of the experiment were raised separately up to the 14th (grower phase) and 28th day of age (finisher phase), respectively, using feed formulated to meet the nutritional recommendations from Brazilian Table for Poultry and Swine (Rostagno et al., 2005). From 1 to 14 days of age the birds were housed in metabolic cages (1.0 m × 0.4 m) arranged in three batteries of six floors, equipped with electric heating, feeders and nipple drinkers. For the other phases the birds were housed in an experimental facility containing 100 pens of 1.5 m^2 each, lined with wood shavings (5 cm thick), equipped with a tubular feeder and nipple drinkers. During the experiment, lighting was provided 24 h daily. Water and feed were provided *ad libitum*. The maximum and minimum temperatures and relative humidity (RH) were recorded daily, inside the facility. From 1 to 14 days, the temperature varied from 31.5 ± 0.49 to 26.3 ± 0.36 °C and RH from 78 ± 6.6 to 61 ± 2.6%, respectively. In the grower phase (14–28 days) the temperatures ranged from 28.2 ± 0.64 to 21.3 ± 0.14 °C and RH from 91 ± 2.5 to 70 ± 3.5%. In the finisher phase, the ranges were from 30.7 ± 0.48 to 21.0 ± 0.16 °C and from 91 ± 1.8 to 58 ± 1.7%, respectively.

Total amino acids content of the ingredients used in the formulation were analysed using high performance liquid chromatography (HPLC) by Ajinomoto Ltd. For that, samples were hydrolysed for 23 h at 110 °C, after performic acid oxidation. Methionine was transformed into methionine sulfone and the cysteine into cysteic acid. These values were converted to a digestible basis using the digestibility coefficients from Brazilian Tables for Poultry and Swine (Rostagno et al., 2005).

Two diets were formulated, one with a high protein content (D1) and the other protein-free (D7). The summit diet was formulated to contain 1.20 times the requirement for valine recommended in the Brazilian Tables for Poultry and Swine (Rostagno et al., 2005) and the other amino acids were formulated to contain a minimum of 1.4 times the recommendations. This procedure is used to provide a high concentrate protein diet with the valine as the first limiting amino acid at a relative

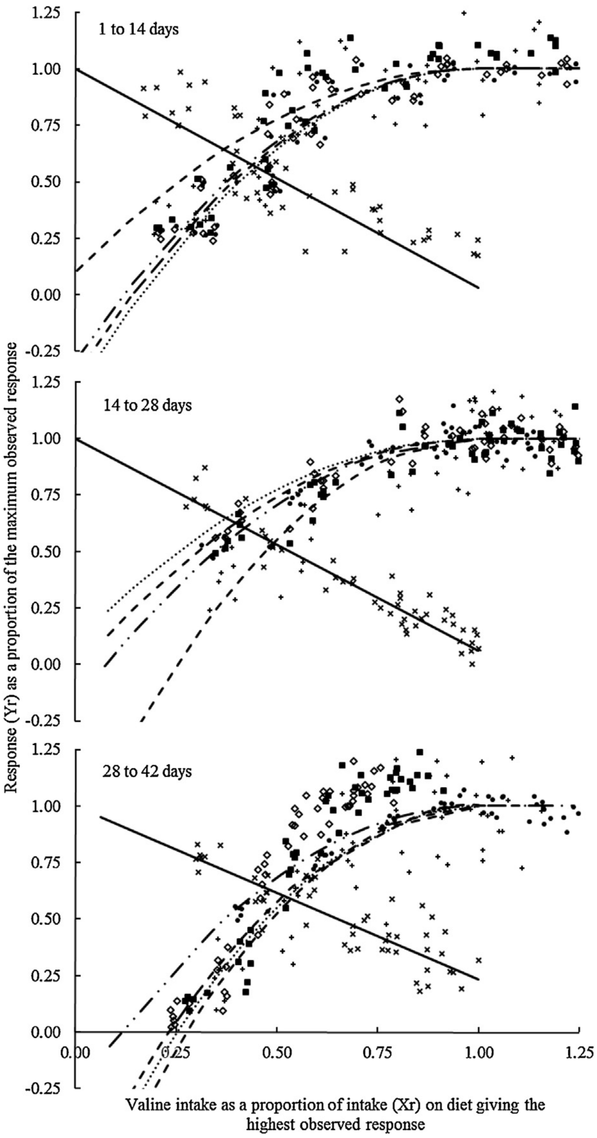

Fig. 1. Relative responses (Yr) to the 95% of asymptotic response model depending on the relative intake of valine (Xr) at the breakpoint of the model for broiler chickens. Lipid content in carcass (× observed, ----- predicted); Protein deposition in feathers (+ observed, - - - predicted); Protein deposition in the carcass (◊ observed, — — — predicted); Deposition valine (■ observed, ... predicted); Weight Gain (● observed, ... predicted) for the three phases.

deficiency of 20% (Fisher and Morris, 1970). The summit and dilution diets were formulated to contain the same levels of energy, minerals and vitamins (Brazilian Table for Poultry and Swine, Rostagno et al., 2005) (Tables 1 and 2). The intermediate levels of digestible valine were obtained by appropriately blending the two basal diets; the proportions used are shown in Table 3.

The control diet (D8) was included to confirm that valine was the first limiting amino acid in the dietary protein. This diet was made by supplementing the diet with the lowest valine content (D7) with L-valine to provide the same level of valine as in D6. The amount of L-valine supplemented was 1.51, 1.57 and 1.42 g/kg in the starter, grower and finisher phase, respectively. The responses obtained to these control diets were not used when fitting models to the data obtained.

2.2. Data collection, sampling, processing and chemical analysis

The birds and feeds were weighed at the start and the end of each trial period to determine average daily weight gain (ADG), feed intake (FI) and feed conversion efficiency (FCE). To determine the protein deposition in the body and feathers the comparative slaughter technique was performed. A representative sample of birds at the beginning (reference groups) of each period was made using 30, 18 and 12 birds at 1, 14 and 28 days, respectively. At the end of each phase (14, 28 and 42 days), two birds per replicate were selected based on average body weight, totalling 112 birds for each phase. The birds were

Table 1
Composition (g/kg) of the summit and dilution diets.

Ingredientes	Summit (valine limiting D1)	Dilution (D7)
Soybean meal	470	–
Corn	370	–
Corn gluten 60%	40.0	–
Soybean oil	52.5	100
Dicalcium phosphate	20.5	27.0
Limestone	9.52	5.07
Salt	10.0	5.13
Choline chloride (60%)	1.00	1.00
Mineral and vitamin premix[1]	0.20	0.20
Methionine hydroxy analog[2]	8.00	–
L-Lysine Hcl (99%)	5.24	–
L-Threonine	3.00	–
L-Valine	0.48	–
L-Isoleucine	2.20	–
L-Arginine	2.70	–
L-Tryptophan	4.10	–
Potassium chloride	–	11.4
Corn starch	–	428
Sugar	–	150
Rice husk	–	150
Inert filler[3]	–	121
Salinomycin sodium 12%[5]	0.50	0.50
Growth promoter[6]	0.05	0.05
Antioxidant[4]	0.10	0.10

[1] Content (per kg of the diet)- vit. A. 12,000 IU; vit. D3. 3,500 IU; vit. K. 4.5 mg, vit. E. 40 mg, vit. B1. 2.5 mg, vit. B2. 8 mg, vit. B6. 6 mg, vit. B12. 32 mg; niacin. 45 mg, folic acid. 1.2 mg, calcium pantothenate. 15 mg; biotin. 0.02 mg; iron. 55 mg, zinc. 97 mg; copper. 20 mg, MN. 120 mg; iodine. 1.1 mg, selenium. 0.34 mg; antioxidant. 4.20 mg.
[2] MHA—methionine hydroxy analog—84%.
[3] Inert filler—washed sand.
[4] Butyl hydroxy toluene BHT.
[5] Coxistac.
[6] Avilamycin.

Table 2
Concentration (g/kg) (calculated) of the summit and dilution diets.

Energy and other nutrients	Summit (valine limiting D1)	Dilution (D)
Metabolizable energy. kcal/kg	3050	3050
Crude protein	283 (283)[1]	7.0 (0.6)[1]
Calcium	10.0	10.0
Sodium	2.20	2.20
Phosphorus available	5.00	5.00
Digestible amino acids:	–	–
Methionine + cystine	14.1	–
Methionine	10.7	–
Lysine	17.9	–
Tryptophan	4.32	–
Threonine	12.4	–
Arginine	20.3	–
Valine	11.5	–
Isoleucine	12.9	–
Leucine	23.3	–
Phenylalanine + tyrosine	20.8	–

Numbers in parentheses refer to the composition analysed.
[1] Crude protein, CP = N × 6.25.

fasted for 36 h to empty the digestive tract. They were then weighed and sacrificed by CO_2 asphyxiation. Representative samples of feathers of back, wings and breast from each bird were collected and carcasses were completely defeathered. The defeathered carcasses were weighed, then minced, and a sample of each whole defeathered carcass was placed in a freezer (−20 °C) prior to further handling. Frozen samples were placed in a freeze-dryer (LABCONCO FREEZE DRYER 5) at a temperature of −80 °C for 72 h for pre-drying. The fat content of each freeze-dried sample was measured (AOAC, 1995 n.984.13) by extraction with petroleum ether in a fat extractor (Ankon Technology®). Total nitrogen content of the defatted carcass and feather samples were measured using the Kjeldahl method (AOAC, 1995 n.984.13), corrected to 100% dry matter (AOAC, 1995 n. 984.13).

Table 3
Mixing proportions of summit and dilution diets and calculated concentration of limiting amino acid.

Diets	1–14 days			14–28 days			28–42 days		
	Summit D1	Dilution D	Valine	Summit D1	Dilution D	Valine	Summit D1	Dilution D	Valine
	%	%	g/kg	%	%	g/kg	%	%	g/kg
D1	100	0	11.5	90	10	10.3	83	17	9.5
D2	88	12	10.1	79	21	9.1	73	27	8.4
D3	76	24	8.7	68	32	7.8	63	37	7.2
D4	64	36	7.4	58	42	6.7	53	47	6.1
D5	52	48	6.0	47	53	5.4	43	57	4.9
D6	40	60	4.6	36	64	4.1	33	67	3.8
D7	28	72	3.2	25	75	2.9	23	77	2.6
D8	28	72	4.6[1]	25	75	4.1[2]	23	77	3.8[3]

Control diet.
[1] D8 = D7 + 1.51 g L-valine/kg.
[2] D8 = D7 + 1.57 g L-valine/kg.
[3] D8 = D7 + 1.42 g L-valine/kg.

Table 4
Mean (±standard error) feed intake, valine intake, body weight gain and feed conversion efficiency (FCE) of broiler chickens submitted to different digestible valine levels in the diet from 1 to 14, 14 to 28, 28 to 42 days of age.

Diet	Digestible valine content (g/kg)	Valine intake (mg/d)	Feed intake (g/days)	Body weight gain (g/days)	FCE (g gain/kg food)
1–14 days					
D1	11.5	235.91 ± 19.87	24.40 ± 1.41	23.59 ± 0.28	1091.67 ± 116.21
D2	10.1	233.06 ± 6.29	25.69 ± 0.69	23.17 ± 0.19	906.21 ± 27.70
D3	8.7	180.05 ± 13.05	23.06 ± 1.67	23.08 ± 0.31	1035.38 ± 80.61
D4	7.4	151.16 ± 11.71	22.70 ± 1.76	20.88 ± 0.24	965.12 ± 83.95
D5	6.0	125.71 ± 4.65	23.29 ± 0.86	17.58 ± 0.75	756.88 ± 26.99
D6	4.6	94.21 ± 6.55	22.79 ± 1.59	11.46 ± 0.20	523.41 ± 43.15
D7	3.2	62.33 ± 4.59	21.71 ± 1.60	6.66 ± 0.14	320.06 ± 26.50
D8	4.6[1]	92.28 ± 5.98	22.32 ± 1.45	7.70 ± 0.03	356.91 ± 26.51
14–28 days					
D1	10.3	757.20 ± 4.63	73.27 ± 0.45	53.17 ± 0.57	726.05 ± 10.97
D2	9.1	716.51 ± 14.60	78.98 ± 1.61	54.87 ± 1.49	665.67 ± 9.77
D3	7.8	640.20 ± 5.41	81.99 ± 0.69	55.06 ± 0.77	671.54 ± 7.26
D4	6.7	622.58 ± 14.15	93.48 ± 2.12	55.97 ± 0.81	600.08 ± 10.69
D5	5.4	523.04 ± 14.74	96.91 ± 2.73	51.40 ± 0.69	547.86 ± 10.24
D6	4.1	373.07 ± 4.58	90.25 ± 1.11	40.95 ± 0.61	453.91 ± 6.06
D7	2.9	250.45 ± 12.84	87.24 ± 4.47	28.15 ± 0.63	327.17 ± 14.65
D8	4.1[2]	391.70 ± 17.31	94.75 ± 4.19	28.98 ± 0.41	331.51 ± 8.71
28–42 days					
D1	9.5	1456.04 ± 21.66	152.77 ± 2.27	81.59 ± 1.14	534.77 ± 9.88
D2	8.4	1353.53 ± 7.10	161.47 ± 0.85	85.90 ± 1.19	532.10 ± 8.09
D3	7.2	1219.67 ± 7.38	168.60 ± 1.02	87.444 ± 1.24	518.67 ± 6.87
D4	6.1	1086.41 ± 10.89	178.51 ± 1.79	88.49 ± 0.92	495.74 ± 2.24
D5	4.9	915.49 ± 6.17	185.41 ± 1.25	80.99 ± 0.71	436.80 ± 2.10
D6	3.8	725.28 ± 7.27	191.40 ± 1.92	67.19 ± 1.26	350.98 ± 4.94
D7	2.6	492.28 ± 11.29	186.39 ± 4.27	46.77 ± 0.80	251.71 ± 6.50
D8	3.8[3]	685.28 ± 10.12	180.84 ± 2.67	47.14 ± 1.42	260.41 ± 5.18

Control diet.
[1] D8 = D7 + 1.51 g L-valine/kg.
[2] D8 = D7 + 1.57 g L-valine/kg.
[3] D8 = D7 + 1.42 g L-valine/kg.

2.3. Calculations and modelling of the responses

Digestible valine intake (mg/bird days) was calculated from the feed intake data and the digestible valine levels analysed. The valine intake for maximum weight gain, valine deposition and protein deposition in the defeathered body and feather were estimated using a broken-line model with quadratic ascension:

$$Y = L + U \times (R\text{-}X)^2 \quad (1)$$

where X is the digestible valine intake (mg/bird days) and Y is the response (weight gain, valine deposition or protein deposition in the defeathered body and feather), L is the response estimated at the plateau of the function, that represent 95% of asymptotic response (maximum response), R is the digestible valine intake at the breakpoint and U is the slope of the model.

The relative response (Yr) was obtained by dividing Y by L and the relative valine intake (Xr) was obtained by dividing X by R. These values were plotted to analyse the behaviour of the relative responses to the relative digestible valine intake.

The maximum efficiency of valine utilisation (k) for each phase was obtained by dividing L by R of the estimated parameters for valine deposition equations.

In addition to the broken-line model were adjusted Quadratic (QUAD), LRP + Quadratic (LRP + QUAD), Exponential, Sigmoid curve and Saturation Kinetic Model (SKM) models, as described below:

$$\text{QUAD} - \text{Y} = \text{AVal}^2 + \text{BVal} + \text{C} \quad (2)$$

where C is the constant regression or intercept, B is the regression parameter for linear component and A is the regression parameter for the quadratic component.

$$\text{LRP} + \text{QUAD} - \text{Y} = \text{L} + \text{U} \times \text{Val} + \text{A} \times \text{Val}^2 \quad (3)$$

where: L is the variable value estimated on the plateau, U is the slope of the ascending line, A is the regression parameter for the quadratic component.

$$\text{Exponential—Y} = \text{A} + \text{B}(1\text{-e}^{-\text{C} \times (\text{X}-\text{D})}) \quad (4)$$

where A is the response for weight gain estimated for the feed with the lower intake of valine; B is the difference estimated between the minimum and maximum response obtained by the increasing valine intake; C is the slope of the exponential curve; D is the valine intake at the lower level (54.1); and e is the base number of natural logarithm (2.718282).

$$\text{Sigmoid—Y} = \text{P} + \text{Q}/(1 + \text{e}^{(\text{r}+\text{s}*\text{Val})}) \quad (5)$$

where P is an upper asymptote, P + Q, and continuous derivatives, Q, r and s for the sigmoid curve.

$$\text{SKM—Y} = (\text{b} \times \text{c} + \text{a} \times \text{Val}^{\text{d}})/(\text{c} + \text{Val}^{\text{d}}) \quad (6)$$

where a is the asymptotic or maximum response; b is the intercept on the y axis, c is Intake is required for half-maximal response; d is apparent kinetic order

The fat content of the body was calculated by dividing the proportion of fat in the body by the body weight. Thereafter, a linear regression was used to adjust the fat content data to the digestible valine intake as follows:

$$Y = \beta_0 + \beta_1 \times (\text{X}) \quad (7)$$

where Y is the relative response, in this case fat content; β_1 is the slope of the linear regression, β_0 is the intercept at y-axis and X is the relative intake of digestible valine (mg/bird d). In this case, to obtain Yr, the value of Y (fat content) was divided by β_0 and Xr was obtained by dividing the valine intake to obtain Y by the maximum intake observed.

2.4. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (Statistical Analysis System, version 9.0). The models were fitted using the PROC NLIN procedure to converge the models at a possible solution. The coefficient of determination (R^2) was obtained as SQmodel/SQtreatment.

3. Results

3.1. Calculations and modelling of responses

Mean feed intake, valine intake, body weight gain and FCE are shown in Table 4, and the mean rates of deposition of protein in the defeathered body and the feathers, fat content and valine deposition in the body are presented for the different levels of digestible valine used in Table 5. The results confirmed that valine was the limiting amino acid in the experimental diets, as all parameter values for broilers fed the control diet (D8) were between to those obtained for the D6 and D7 diets.

There was an improvement in body weight gain and feed intake with increasing dietary intake of valine in the three experimental phases and, consequently, an improvement in FCE.

Regarding of the models (Table 6), among the equations adjusted in the manuscript, was chose the broken-line, because had good fit and the parameters have biological significance which is very important to discuss the results. Also opted to use only one model for all variables, to standardize the description of the responses.

The amount of protein and valine deposition in the defeathered carcass, and feathers was positively associated with valine intake. On the other hand, in those diets with reduced valine levels, hence lower protein and amino acid concentration, the fat content of the carcass increased. The magnitude of change in the fat content of the carcass was positively and directly related to voluntary feed intake, which increased to compensate for the reduced levels of dietary valine. Body weight gain, deposition of protein in the defeathered body and feathers, and body fat content adjusted by the broken-line and linear regression as a function of valine intake are presented in Table 6.

The digestible valine intake for maximum weight gain in the three phases of growth was estimated at 218, 678 and 1174 mg/bird days, respectively (Table 7). For maximum deposition of protein in the defeathered body, intakes were 222, 614

Table 5
Effect of valine levels on mean live weight (kg), feather free body weight (kg) and body composition (g/kg feather free body weight) of Cobb broiler used in trials using comparative slaughter technique.

	Live weight	Feather free body weight	Water	Protein	Lipid	Ash
RS1	0.039	0.038	191.4	124.8	30.8	35.8
RS2	0.264	0.257	260.4	148.3	63.6	48.5
RS3	1.103	1.026	424.2	244.7	122.1	57.4
14 days						
D1	0.318	0.308	242.1	152.7	37.4	51.9
D2	0.315	0.306	260.9	155.3	51.1	54.5
D3	0.313	0.304	270.6	155.2	68.3	47.1
D4	0.301	0.292	284.2	151.9	87.2	45.1
D5	0.255	0.247	302.6	152.6	108.1	41.9
D6	0.176	0.171	334.9	157.0	149.9	28.0
D7	0.125	0.121	338.9	147.3	168.8	22.8
D8	0.134	0.131	351.1	150.4	158.5	42.2
28 days						
D1	0.884	0.838	265.1	162.7	40.8	61.6
D2	0.902	0.864	279.0	161.2	65.8	52.1
D3	0.915	0.876	289.2	161.1	72.7	55.4
D4	0.903	0.866	307.3	165.8	89.5	52.0
D5	0.866	0.834	325.4	169.7	117.8	37.8
D6	0.741	0.717	348.3	168.4	148.2	31.6
D7	0.589	0.568	374.8	178.3	183.2	13.3
D8	0.594	0.578	356.4	168.7	162.6	26.6
42 days						
D1	2.215	2.032	440.0	270.2	125.9	44.0
D2	2.310	2.115	440.0	254.1	137.4	48.5
D3	2.322	2.131	440.0	245.3	157.2	37.5
D4	2.321	2.127	440.0	237.6	175.7	26.7
D5	2.257	2.076	397.6	207.6	178.5	11.4
D6	2.034	1.870	350.0	169.4	168.3	12.3
D7	1.806	1.668	342.7	163.1	177.4	2.2
D8	1.812	1.681	343.3	164.0	179.0	3.1

RS1 = reference slaughter with 1 day old.
RS2 = reference slaughter with 14 days old.
RS3 = reference slaughter with 28 days old.
D1–D8 = diet.

and 1976 mg/bird days, respectively, and for maximum deposition of protein in the feathers, 235, 640 and 1351 mg/bird days. The equations were adjusted appropriately to the data ($P < 0.001$), pointing to the suitability of the model for representing the biological behavior of the parameters.

For maximum deposition of valine in the body, the valine intake was estimated at 227, 614 and 1711.6 mg/bird days for the starter, grower and finisher phase, respectively. Therefore, the relation between maximum deposition predicted for valine (L) and intake for maximum valine deposition (R), resulted in an efficiency of valine utilization (k) of 0.80 ($k = 182/227$) for the starter phase, 0.65 ($k = 400/614$) for the grower, and 0.61 ($k = 1046/1711.6$) for the finisher phase of the trial.

Overall, the results show that estimated weight gain, protein deposition in the defeathered body and the feathers, body fat content and valine deposition in the defeathered body values were similar within each experimental phase, with average estimated digestible valine requirement equal to 226, 637 and 1553 mg/bird d from in the three phases, respectively.

To compare the response of the different parameters measured, daily valine intake was standardized as a proportion of the feed intake corresponding to the maximum predicted response for each parameter. Fig. 1 is observed the relative responses to the 95% of the asymptotic response model for weight gain, deposition of protein in the defeathered body and the feathers and fat content in the body are compared to those predicted as a function of relative intake of digestible valine for the three phases of growth.

Weight gain and deposition of protein in the defeathered body were the parameters most affected by valine deficiency, which in all phases they were negative for intakes below 0.25 of intake in the diet yielding maximum response at breakpoint (R). For all phases, a linear decrease in the proportion of maximum fat content was observed with the increase in digestible valine intake.

4. Discussion

This study aimed to measure the response in performance and body composition of broiler chickens subjected to a range of digestible valine intakes. The improved response observed from the addition of valine to the low protein diet (D7 *vs.* D8) (Tables 5 and 6) confirmed that valine was the first limiting amino acid in the feeds used in our trials. This improvement confirmed that the relative amino acid deficiency was independent of dietary protein level and was not influenced by the

Table 6
Mean (±standard error) rates of protein deposition in the body, in feathers and valine deposition in body and feathers, and body fat content of broiler chickens submitted to different digestible valine levels in the diet from 1 to 14, 14 to 28, 28 to 42 days of age.

Diet	Valine level (g/kg)	Protein deposition in the body (g/days)	Protein deposition in feathers (g/days)	Valine deposition (mg/days)	Fat content (g/kg)
1–14 days					
D1	11.5	3.61 ± 0.07	0.64 ± 0.03	197.43 ± 4.41	36.24 ± 2.07
D2	10.1	3.60 ± 0.03	0.63 ± 0.03	195.95 ± 1.47	49.53 ± 1.47
D3	8.7	3.60 ± 0.05	0.55 ± 0.03	191.39 ± 2.75	70.69 ± 1.64
D4	7.4	3.17 ± 0.03	0.55 ± 0.03	172.53 ± 2.83	87.19 ± 1.41
D5	6.0	2.69 ± 0.12	0.45 ± 0.01	145.25 ± 5.93	105.26 ± 3.62
D6	4.6	1.80 ± 0.05	0.28 ± 0.02	96.36 ± 2.89	149.64 ± 6.14
D7	3.2	0.98 ± 0.03	0.20 ± 0.01	55.95 ± 1.48	168.17 ± 4.92
D8	4.6[1]	1.07 ± 0.02	0.20 ± 0.01	61.84 ± 0.85	158.21 ± 10.28
14–28 days					
D1	10.3	7.02 ± 0.09	1.44 ± 0.18	394.84 ± 10.82	26.14 ± 3.87
D2	9.1	7.22 ± 0.16	1.17 ± 0.11	388.13 ± 12.11	53.28 ± 4.45
D3	7.8	7.36 ± 0.28	1.24 ± 0.11	398.52 ± 6.68	61.84 ± 5.59
D4	6.7	7.53 ± 0.14	1.11 ± 0.05	398.10 ± 7.96	78.01 ± 3.40
D5	5.4	7.40 ± 0.14	0.95 ± 0.04	382.42 ± 14.03	113.53 ± 4.25
D6	4.1	5.91 ± 0.20	0.71 ± 0.05	302.28 ± 8.28	156.62 ± 5.43
D7	2.9	4.30 ± 0.12	0.41 ± 0.03	217.90 ± 6.48	223.24 ± 7.29
D8	4.1[2]	4.36 ± 0.07	0.42 ± 0.07	214.90 ± 5.59	193.13 ± 9.58
28–42 days					
D1	9.5	21.28 ± 0.37	3.91 ± 0.23	1170.99 ± 21.8	97.28 ± 10.31
D2	8.4	20.46 ± 0.68	4.30 ± 0.25	1158.34 ± 15.9	121.08 ± 16.98
D3	7.2	19.40 ± 0.81	4.19 ± 0.27	1105.30 ± 18.8	139.55 ± 9.55
D4	6.1	18.16 ± 0.52	3.96 ± 0.44	1036.41 ± 45.9	144.25 ± 7.19
D5	4.9	12.86 ± 0.50	3.42 ± 0.19	770.92 ± 35.6	215.90 ± 10.83
D6	3.8	4.70 ± 0.50	2.16 ± 0.23	336.51 ± 36.6	201.49 ± 9.43
D7	2.6	1.49 ± 0.37	0.94 ± 0.11	122.13 ± 15.16	260.83 ± 4.88
D8	3.8[3]	1.76 ± 0.24	0.91 ± 0.22	131.49 ± 17.00	250.87 ± 7.82

Control diet.
[1] D8 = D7 + 1.51 g L-valine/kg.
[2] D8 = D7 + 1.57 g L-valine/kg.
[3] D8 = D7 + 1.42 g L-valine/kg.

dilution technique. According to Fisher and Morris (1970) this is an essential component of a response trial as a means of confirming that the amino acid under test is first-limiting in the dilution series.

As expected, there was an improvement in body weight gain and feed intake with an increase in digestible valine intake in the three phases (Table 5). As a consequence, FCE also improved, as observed in Gous and Morris, 1985; Corzo et al., 2004. This improvement in FCE is related to the increasing efficiency of valine utilization for body protein synthesis with greater valine intake and, consequently, higher protein deposition (Table 6). This is due to the capacity of valine and other branched chain amino acids to change muscle protein degradation (Chua et al., 1979; Morgan et al., 1981).

Consumption of valine deficient diets has been associated with feather and leg abnormalities (Farran and Thomas, 1992a,b). In our study, feather abnormalities were also observed and were associated with the reduced deposition of protein caused by the valine deficiency (Table 6) which could have altered the proportion of amino acids other than the branched-chain amino acids in the feathers (Farran and Thomas, 1992a). The response on the deposition of feather protein was more expressive in the growth phase because it is during this phase that occurs at higher deposition of feathers (Marcato et al., 2009).

Knowledge of the response to valine not only allows for an increase in protein deposition in the body and feathers but also for a reduction in the fat content of the carcass (Burnham et al., 1992; Choct et al., 2005). In the present study, it is clear that decreased valine intake is accompanied by increased body fat deposition (Fig. 1). This is not surprising since a decrease in the dietary amino acid content results in an overconsumption of energy which is then deposited in the body as fat, as observed in previous studies in which the dilution technique has been used to measure the response of broilers to other amino acids (Gous and Morris, 1985; Burnham et al., 1992).

The response curve describing the rates of deposition of valine and protein in the defeathered body and feathers, as well as weight gain, followed the same basic shape (Fig. 1). Previous research has indicated that the shape of these response curves, and the estimated plateau, are highly influenced by the range of amino acid supplementation used (Rodehutscord and Pack, 1999). From a biological perspective, the shape of the relationship between valine intake and the parameters observed is indeed more representative of the physiological processes that take place during growth, since there is a nonlinear relationship between amino acid intake and the corresponding increase in efficiency of utilization (Gahl et al., 1994; Rodehutscord et al., 1997; Fatufe et al., 2004).

Table 7
Models fitted for weight gain, protein deposition and valine deposition rate in the defeathered body and feathers, and fat content adjusted by the linear regression methods, as a function of digestible valine intake for broilers from 1 to 14 (1 phase), 14–28 (2 phase) and 28–42 days (3 phase) of age.

Equation	Weight gain	R^2	SEE	RPE	CCC	P > F
	1 phase					
Broken-line	$Y = 23.5 + (-0.0006)*(218\text{-Val})^2$	0.85	0.56	1.00	1.00	0.0001
LRP + QUAD	$Y = 23.646–0.0005*(\text{Val}-239.5)^2$	0.80	0.90	0.99	0.99	0.0001
QUAD.	$Y = -4.588 + 0.217\text{Val} - 0.0004\text{Val}^2$	0.60	1.34	0.98	0.98	0.0001
Exponential	$Y = 27.554\text{-}(27.554–5.315)*e^{(-0.0097*(\text{Val}-54.1))}$	0.78	1.22	0.98	0.98	0.0001
Sigmoid	$Y = 7.691 + 15.213/(1 + e^{(11.123-0.096*\text{Val})})$	0.86	1.39	0.98	0.98	0.0001
SKM	$Y = 7.072*(3.9E + 14) + 23.406*\text{Val}^{7.079})/((2.73E + 09) + \text{Val}^{7.079}$	0.85	137.20	−0.79	−0.79	0.0001
	2 phase					
Broken-line	$Y = 54.75 + (-0.00014)*(678\text{-Val})^2$	0.92	0.72	1.00	1.00	0.0001
LRP + QUAD	$Y = 12.33 + 0.00014*(\text{Val-}677.9)^2$	0.90	0.72	−1.00	−1.00	0.0001
QUAD.	$Y = -12.33 + 0.201\text{Val-}0.00015*\text{Val}^2$	0.90	0.71	1.00	1.00	0.0001
Exponential	$Y = 58.265\text{-}(58.265–17.248)*e^{(-0.0049*(\text{Val}-54.1))}$	0.88	0.94	0.99	0.99	0.0001
Sigmoid	$Y = 25.727 + 28.888/(1 + e^{(7.100-0.019*\text{Val})})$	0.90	1.30	0.99	0.99	0.0001
SKM	$Y = 23.494*(9.80E + 11) + 56.447*\text{Val}^{4.688})/((9.80E + 11) + \text{Val}^{4.688}$	0.90	1.36	0.99	0.99	0.0001
	3 phase					
Broken-line	$Y = 86.9 + (-0.00008)*(1174\text{-Val})^2$	0.94	1.31	1.00	1.00	0.0001
LRP + QUAD	$Y = 85.907 + 0.000084(\text{Val-}1174.1)^2$	0.93	1.37	−1.00	−1.00	0.0001
QUAD.	$Y = -29.090 + 0.193*\text{Val-}0.00008*\text{Val}^2$	0.94	1.32	1.00	1.00	0.0001
Exponential	$Y = 88.170\text{-}(88.170–116.4)*e^{(-0.004*(\text{Val}-54.1))}$	0.90	0.50	−0.97	−0.97	0.0001
Sigmoid	$Y = 43.594 + 42.193/(1 + e^{(8.210-0.012*\text{Val})})$	0.93	2.54	0.99	0.99	0.0001
SKM	$Y = 25.526*(3.80E + 10) + 89.198*\text{Val}^{3.821})/((3.80E + 10) + \text{Val}^{3.821}$	0.91	3.57	0.97	0.97	0.0001
Equation	Protein deposition	R^2	SEE	RPE	CCC	P > F
	1 phase					
Broken-line	$Y = 3.62 + (-0.0001)*(222\text{-Val})^2$	0.86	0.08	1.00	1.00	0.0001
LRP + QUAD	$Y = 3.665–0.00008*(\text{Val-}240.7)^2$	0.80	0.13	0.99	0.99	0.0001
QUAD.	$Y = -4.588 + 0.217*\text{Val-}0.0004*\text{Val}^2$	0.60	1.30	0.98	0.98	0.0001
Exponential	$Y = 4.272\text{-}(4.272–0.774)*e^{(-0.010*(\text{Val}-54.1))}$	0.79	0.18	0.98	0.98	0.0001
Sigmoid	$Y = 1.113 + 2.421/(1 + e^{(9.950-0.086*\text{Val})})$	0.86	0.22	0.98	0.98	0.0001
SKM	$Y = 1.148*(1.018E + 21) + 3.546*\text{Val}^{10.180})/((1.018E + 21) + \text{Val}^{10.180}$	0.86	0.23	0.98	0.98	0.0001
	2 phase					
Broken-line	$Y = 7.3 + (-0.00002)*(614\text{-Val})^2$	0.78	0.11	0.99	0.99	0.0001
LRP + QUAD	$Y = 7.308–0.00002*(\text{Val-}614.1)^2$	0.77	0.11	0.99	0.99	0.0001
QUAD.	$Y = -0.796 + 0.027*\text{Val-}0.0002*\text{Val}^2$	0.80	25.32	−0.76	−0.76	0.0001
Exponential	$Y = 7.491\text{-}(7.491–2.620)*e^{(-0.006*(\text{Val}-54.1))}$	0.74	0.14	0.97	0.97	0.0001
Sigmoid	$Y = 4.462 + 2.849/(1 + e^{(12.035-0.032*\text{Val})})$	0.80	0.18	0.99	0.99	0.0001
SKM	$Y = 4.500*(1.81E + 34) + 7.312*\text{Val}^{13.317})/((1.81E + 34) + \text{Val}^{13.317}$	0.79	0.18	0.99	0.99	0.0001
	3 phase					
Broken-line	$Y = 19.5 + (-0.00001)*(1976\text{-Val})^2$	0.77	1.25	0.98	0.98	0.0001
LRP + QUAD	$Y = 23.805–0.00001\text{Val}*1837.7*\text{Val}^2$	0.92	1.27	0.98	0.98	0.0001
QUAD.	$Y = -20.293 + 0.048*\text{Val-}0.00001*\text{Val}^2$	0.92	1.65	0.98	0.98	0.0001
Exponential	$Y = 36.712\text{-}(36.712–18.514)*e^{(-0.001*(\text{Val}-54.1))}$	0.90	0.57	0.98	0.98	0.0001
Sigmoid	$Y = 0.786 + 20.077/(1 + e^{(7.910-0.009*\text{Val})})$	0.94	0.40	1.00	1.00	0.0001
SKM	$Y = (1.290*(3.97E + 23) + 21.268*\text{Val}^{8.010})/((3.97E + 23) + \text{Val}^{8.010})$	0.94	0.29	1.00	1.00	0.0001
Equation	Feathers protein deposition	R^2	SEE	RPE	CCC	P > F
	1 phase					
Broken-line	$Y = 0.61 + (-0.00001)*(235\text{-Val})^2$	0.71	0.02	0.98	0.98	0.0001
LRP + QUAD	$Y = 0.633–0.00001\text{Val}*268.6\text{Val}^2$	0.67	0.03	−0.85	0.99	0.0001
QUAD.	$Y = -4.588 + 0.217\text{Val-}0.0004\text{Val}^2$	0.44	1.18	0.99	0.99	0.0001
Exponential	$Y = 0.771\text{-}(0.771–0.114)*e^{(-0.008*(\text{Val}-54.1))}$	0.67	0.03	0.98	0.98	0.0001
Sigmoid	$Y = 0.216 + 0.373/(1 + e^{(13.594-0.116*\text{Val})})$	0.74	0.05	0.97	0.97	0.0001
SKM	$Y = (0.200*(9.596E + 14) + 0.609*\text{Val}^{7.242})/((9.596E + 14) + \text{Val}^{7.242})$	0.73	0.03	0.96	0.99	0.0001
	2 phase					
Broken-line	$Y = 1.20 + (-5.3E\text{-}06)*(640\text{-Val})^2$	0.76	0.15	0.89	0.89	0.0001
LRP + QUAD	$Y = 2.655–0.0000005*\text{Val}*2453.5\text{Val}^2$	0.56	0.07	−0.94	0.98	0.0001
QUAD.	$Y = -0.074 + 0.002\text{Val-}0.000004\text{Val}^2$	0.56	0.14	−0.93	−0.93	0.0001
Exponential	$Y = 4.301\text{-}(4.301 + 0.038)*e^{(0.0005*(\text{Val}-54.1))}$	0.56	0.07	−0.98	0.98	0.0001
Sigmoid	$Y = -9.829 + 13.325/(1 + e^{(-1.004-0.009*\text{Val})})$	0.56	0.10	0.90	0.90	0.0001
SKM	$Y = (33.589*(-9.369) + 7.857*\text{Val}^{0.090})/((-9.367) + \text{Val}^{0.090})$	0.55	0.06	0.92	0.98	0.0001
	3 phase					
Broken-line	$Y = 4.20 + (-0.000004)*(1351\text{-Val})^2$	0.71	0.19	0.99	0.99	0.0001
LRP + QUAD	$Y = 4.166–0.0000004*\text{Val}*1350.9\text{Val}^2$	0.70	0.02	−0.79	0.99	0.0001
QUAD.	$Y = -3.945 + 0.012\text{Val-}0.00000004\text{Val}^2$	0.70	1.81	0.92	0.92	0.0001
Exponential	$Y = 4.700\text{-}(4.700–5.568)*e^{(0.0023*(\text{Val}-54.1))}$	0.69	5.13	0.76	0.76	0.0001

Table 7 (*Continued*)

Equation	Weight gain	R^2	SEE	RPE	CCC	P > F
Sigmoid	$Y = 0.743 + 3.412/(1 + e^{(6.789-0.009*Val)})$	0.71	0.14	0.99	0.99	0.0001
SKM	$Y = (-21.032*(-182.5) + 6.226*Val^{1.072})/((-182.5) + Val^{1.072})$	0.68	0.60	−0.97	−0.97	0.0001
Equation	Valine deposition	R^2	SEE	RPE	CCC	P > F
	1 Phase					
Broken-line	$Y = 182 + (-0.005)*(227\text{-Val})^2$	0.85	4.50	1.00	1.00	0.0001
LRP + QUAD	–			NS-		
QUAD.	–			NS		
Exponential	$Y = 4.272\text{-}(4.272-0.774)*e^{(-0.010*(Val-54.1))}$	0.84	1.41	−0.80	−0.80	0.0001
Sigmoid	$Y = 1.113 + 2.421/(1 + e^{(9.950-0.086*Val)})$	0.87	5.39	1.00	1.00	0.0001
SKM	$Y = (1.148*(1.018E+21) + 3.546*Val^{10.180})/((1.018E+21) + Val^{10.180})$	0.82	8.67	0.99	0.99	0.0001
	2 phase					
Broken-line	$Y = 400 + (-0.001)*(614\text{-Val})^2$	0.85	9.43	0.98	0.98	0.0001
LRP + QUAD	$Y = 7.308-0.00002Val*614.1Val^2$	0.82	5.82	1.00	1.00	0.0001
QUAD.	$Y = -0.796 + 0.027Val\text{-}0.00021Val^2$	0.82	5.93	1.00	1.00	0.0001
Exponential	$Y = 7.491\text{-}(7.491-2.620)*e^{(-0.0064*(Val-54.1))}$	0.79	10.85	0.99	0.99	0.0001
Sigmoid	$Y = 4.462 + 2.849/(1 + e^{(12.035-0.032*Val)})$	0.83	5.46	1.00	1.00	0.0001
SKM	$Y = (4.500*(1.81E+34) + 7.312*Val^{13.317})/((1.81E+34) + Val^{13.317})$	0.83	5.22	1.00	1.00	0.0001
	3 phase					
Broken-line	$Y = 1046 + (-0.001)*(1712\text{-Val})^2$	0.82	65.26	0.98	0.98	0.0001
LRP + QUAD	$Y = 23.805-0.00001Val*1837.7Val^2$	0.93	81.93	0.98	0.98	0.0001
QUAD.	$Y = -20.293 + 0.048Val\text{-}0.000013Val^2$	0.93	82.40	0.98	0.98	0.0001
Exponential	$Y = 36.712\text{-}(36.712-18.514)*e^{(-0.00096*(Val-54.1))}$	0.92	89.62	0.98	0.98	0.0001
Sigmoid	$Y = 0.786 + 20.077/(1 + e^{(7.910-0.009*Val)})$	0.96	11.11	1.00	1.00	0.0001
SKM	$Y = (1.290*(3.97E+23) + 21.268*Val^{8.010})/((3.97E+23) + Val^{8.010})$	0.96	6.41	1.00	1.00	0.0001
Fat content						
Parameters	1 phase	2 phase	3 phase			
β_0	191 ± 15.0	298 ± 15.0	334 ± 17.0			
β_1	−0.6161 ± 0.0007	−0.28 ± 0.0007	−0.16 ± 0.014			
R^2	0.77	0.92	0.72			
P > F	0.0001	0.0001	0.0001			

Models used in SAS to adjust the data.
Broken-line—$Y = L + U \times (R \times Val)$; LRP + QUAD—$Y = Y = L + UVal + AVal^2$; QUAD.—$Y = AVal^2 + BVal + C$; Exponential—$Y = Y = A + B(1\text{-}e\text{-}C \times (X\text{-}D))$; Sigmoid—$Y = P + Q/(1 + e^{(r+s*Val)})$; SKM—$Y = b \times c + a \times Val^d / c + Val^d$; Fat content—$Y = \beta_0 + \beta_1 \times (X)$.
SEE (standard error of the estimate), Relative prediction error (RPE) and the concordance correlation coefficient (CCC).

In the present study, it was chosen broken-line for assessing the responses a function of the valine consumption, since in literature review, it is possible to find reference papers, as Baker et al., 2002, in which evaluated the poultry response to intake of amino acids using the broken-line. In addition to, the authors reported that the broken-line is a good model, because it is possible to estimate the minimum requirement needed to achieve the maximum response (Baker, 2003). When the response parameters studied were standardized based on the valine intake, 95% of the asymptotic response (maximum response), was found that the optimum requirement estimated for different responses was similar. This pattern was also described by Leclercq (1998) in his study of valine requirements in broilers, whereas for other essential amino acids the estimated requirements have been shown to vary with the response criterion (Timmler and Rodehutscord, 2003). On top of that, since each phase was conducted separately (without reuse birds), the effect of the period in the grower phase can affect the response in the next phase, implying in a possible limitation of the results using the present procedure to evaluate valine intake.

The maximum efficiency of valine utilisation in this study was 80%, 65% and 61% in the three experimental phases, while an overall efficiency of only 49% had been previously observed (Timmler and Rodehutscord, 2003). This difference is possibly related to the method used to measure the response, to the higher supplementation of valine in our study, as well as to their use of the synthetic form of valine (L-valine), which is highly digestible.

In conclusion, our results indicate that to maximize performance of broiler chickens across several parameters, the recommended intake of digestible valine for the starter, grower and finisher diet is 226, 637 and 1553 mg/bird days, respectively. In order to implement such recommendations these intakes must be converted to dietary valine concentrations, which would require an estimate to be made of the amount of food the broilers would consume in each of the three phases: those estimates must account for the genotype, the environment in which the broilers are to be housed, and the composition of the food to be provided, as each of these factors will influence the daily amount of food consumed by broilers. Simulation models that are available that are capable of predicting food intake under different circumstances which also provide an alternative means of determining the optimum economic amino acid contents to be provided at different stages of growth (Gous, 2013, 2014; EFG Software, 2014). Therefore, the optimum dietary valine content will also depend on the way the birds are sold (whole chicken or cut-up) and age of the birds at slaughter.

Conflict of interest

None.

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