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The optimal digestible valine, isoleucine and tryptophan intakes of broiler breeder hens for rate of lay

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ABSTRACT

Three concurrent assays were conducted with objective of to evaluate the response of broiler breeder hens to valine (Val), isoleucine (Ile), and tryptophan (Trp) intake, determine amino acid utilization efficiency (k), and develop a factorial model. One hundred ninety-two hens were used in each amino acid (AA) assay. A completely random design was used, which consisted of eight treatments, eight replicates, and one hen per cage. The diets were formulated by dilution technique using one summit diet and one nitrogen (N)-free diet, resulting in AA levels that ranged from 1.90 to 9.52, 1.75 to 8.75, and 0.52 to 2.59 g/kg of Val, Ile, and Trp, respectively. A validating diet was included for each amino acid studied to confirm that the response of the birds was a function of the limiting amino acid. Each experiment lasted nine weeks (five weeks of adaptation and four weeks for data collection). The data obtained were AA intake (AAI), body weight (BW), and egg output (EO). Broken line model was used to evaluate the responses. The model design used was $AAI = [AA_m \times (BW \times 0.196)^{0.73}] + [(N_{egg} \times EO \times AA_{egg})/k]$, where AA_m is AA for maintenance (247, 134, or 37 mg/$BP_m^{0.73}$ for Val, Ile, and Trp respectively); $BP_m^{0.73}$ is mature body protein or $(BW \times 0.196)^{0.73}$; k is 0.70 for Val, 0.66 for Ile, or 0.55 for Trp; N_{egg} is the N content in the egg (1.89 g/100 g); and AA_{egg} is the AA content in the egg (413, 338, or 108 mg/g for Val, Ile, and Trp respectively). The additional response seen with the supplementation of the crystalline amino acid confirmed that Val, Ile, and Trp were the first limiting amino acid. The values estimated by the model for utilization efficiency were: 70, 66, and 55% for Val, Ile, and Trp, respectively. The AAI estimated by the model at 30 weeks was 803, 708, and 232 mg/day for Val, Ile, and Trp, respectively. The prediction of the model was improved using the coefficients estimated here with physiologically relevant units.

1. Introduction

Constant genetic improvements have enhanced poultry industry productivity in recent decades. In parallel with these advances, feed producers must frequently redefine nutritional plans considering new breed requirements, scientific developments, and local or global economic situation of the productive chain. Despite existing recommendations (NRC, 1994; Rostagno et al., 2011), industry

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Abbreviations: AA, amino acids; AAI, amino acid intake; BW, body weight; BWG, body weight gain; E, efficiency; EO, egg output; Ile, Isoleucine; RL, rate of lay; Trp, Triptofano; Val, valine

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professionals often attempt to improve rate of lay, egg weight, and hatchling weight by manipulating protein daily intake with no attention to specific amino acid requirements. Nevertheless, a number of reports suggest that high protein intake negatively affects fertility and hatchability (Pearson and Herron, 1982; Shafey, 2002; Ekmay et al., 2013). In addition, increasing the intake of amino acids such as lysine and isoleucine adversely affects hen fertility and egg hatchability (Ekmay et al., 2013).

Mathematical models represent an important tool that incorporates productivity variables to update nutritional requirements. These models also allow for greater flexibility than experimental methods in determining what variables might be more relevant to specific regions or even individual producers (Gous, 2014). However, to have good predictive value models must incorporate the correct factors and coefficients. Furthermore, these factors and coefficients must often be adjusted to new breeds and conditions.

Specifically regarding broiler breeder hens, existing factorial models mostly rely on coefficients and data from commercial laying hens. This is clearly not an optimal situation. For example, laying hens have an amino acid utilization efficiency of 85% whereas for broiler breeders this number is closer to 49% (Bowmaker and Gous, 1991; Silva et al., 2015a). These differences arise from the egg-laying potential of the two types of hens, and demonstrate how poorly data from one breed, inserted into a model, would predict nutritional needs of the other.

Therefore, more studies are necessary to define the relevant variables and correct coefficients to be used in mathematical models for the nutrition of broiler breeders. Thus, in this study we evaluated the response of broiler breeder hens to different concentrations of valine, isoleucine, and tryptophan, three amino acids that are rarely studied in this context. We determined amino acid utilization efficiency and adapted the mathematical model originally developed by Bornstein et al. (1979).

2. Materials and methods

2.1. Birds and experimental design

Three dose response studies were performed using broiler breeder hens. The Animal Ethics and Welfare Committee of Universidade Estadual Paulista approved all experimental procedures used in this study under the Protocol number 9999/14.

One hundred ninety-two broiler breeder hens of Cobb 500® genotype with 48 weeks of age were used, with sixty-four birds per trial housed individually in metabolic cages. The cages were equipped with individual feeders and nipple drinkers. The experimental design was a completely randomized design with eight treatments (seven increasing levels of the amino acid and a control diet) and eight replicates.

2.2. Bird management

Two weeks before the beginning of the experiment, all birds were fed 150 g per day of a diet designed to meet their nutritional requirements during this period, according to recommendation of Rostagno et al. (2011). The rate of lay was monitored to provide a baseline for the experimental period. The experiment lasted nine weeks, with the first five weeks of adaptation and the last four weeks for data collection.

The lighting program adopted during the experiment was 17 hours of light. The hens were raised in a poultry house with a negative-pressure system with controlled temperature of 21 °C. The daily management was performed according to the Cobb 500 guidelines.

2.3. Experimental diets

For each amino acid assay a high protein summit diet, based on corn and soybean meal, was formulated, containing 9.52 g/kg of Val, 8.75 g/kg of Ile, and 2.59 g/kg of Trp (Table 1). The total amino acid content in experimental diets were analysed by Ajinomoto using high-performance liquid chromatography (HPLC), and values obtained were corrected for digestible amino acids using the tabulated coefficients of digestibility (Rostagno et al., 2011).

For each trial a nitrogen-free diet was formulated to meet the same nutritional levels as the summit diets, except for protein and amino acids. The nitrogen-free diets were used to dilute the summit diets, in appropriate proportions, to obtain the range of Val, Ile, and Trp (Table 2) contents required for each dilution series (Fisher and Morris, 1970).

To confirm that the response of the birds to each dilution series was in function of the respective limiting amino acid, a control diet was included for each amino acid assay. A small quantity of the respective crystalline amino acid was added to the diet with the lowest level of the amino acid tested sufficient to meet the level of the amino acid in the second-lowest level in the dilution series (Table 2).

2.4. Allocation of diets and measurements

The birds were fed 150 g per day of feed, at the same time each morning, and at the end of the week the leftovers were weighed to quantify the weekly consumption of the feed. The body weight of the hens was measured on the first, sixth, and tenth weeks of the assay. Egg production was recorded daily and egg weight was measured on three consecutive days each week.

Table 1
Composition (g/kg) of the summit (high protein) and nitrogen-free (N-free) diets used in the valine, isoleucine, and tryptophan response trials.

Ingredients	Valine summit	Isoleucine summit	Tryptophan summit	N-free
Corn	442.6	414.4	494.8	–
Corn gluten meal 60%	–	–	10.0	–
Soybean meal	428.7	453.6	372.6	–
Corn starch	–	–	–	516.9
Rice husk	–	–	–	150.0
Sugar	–	–	–	150.0
Soybean oil	27.1	32.9	14.1	40.0
Limestone	66.7	66.7	66.7	61.9
Dicalcium phosphate	15.1	14.9	15.6	21.6
Salt	5.9	5.9	5.9	5.9
Potassium chloride	–	–	–	18.1
DL-methionine (99%)	5.0	4.8	5.3	–
L-Lysine HCl (78%)	1.0	0.2	2.7	–
L-Threonine	2.6	2.3	3.2	–
L-Tryptophan	0.2	0.1	–	–
L-Arginine	–	–	1.0	–
L-Valine	–	1.3	2.4	–
L- Isoleucine	2.1	–	2.8	–
Choline chloride 60%	1.0	1.0	1.0	1.0
Vitamin premix[a]	1.0	1.0	1.0	1.0
Trace premix[b]	1.0	1.0	1.0	1.0
BHT[c]	0.1	0.1	0.1	0.1
Inert (Sand)	–	–	–	32.4
Total	1000	1000	1000	1000

	Nutrient content[d] (Digestible amino acid composition)						
	Calculated	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Crude protein, g/kg	241.50	265.70	249.09	271.60	231.99	275.80	0.14
	7.92	5.46	7.82	5.32	8.07	5.84	–
Methionine + cysteine	11.11	8.30	11.11	8.10	11.11	8.90	–
Lysine	12.77	12.37	12.77	11.14	12.77	12.78	–
Threonine	10.34	8.33	10.34	7.83	10.34	8.71	–
Tryptophan	2.94	2.66	2.94	2.61	2.50	2.59	–
Arginine	15.18	13.74	15.89	13.66	14.68	15.01	–
Valine	9.85	9.52	11.49	9.47	11.49	10.20	–
Isoleucine	11.49	10.48	9.85	8.75	11.49	10.36	–
Leucine	17.96	17.57	18.51	16.57	17.67	17.95	–

[a] Content per kg diet: vitamin A, 9.000 IU; vitamin D3, 2.600 IU; vitamin E, 14 IU; vitamin K3, 16 mg; vitamin B1, 22 mg; vitamin B2, 6 mg; vitamin B6, 3 mg; vitamin B12, 10 mcg; nicotinic acid, 0.03 g; pantothenic acid, 0.15 g; folic acid, 0.6 mg; biotin, 1 mg.

[b] Content per kg diet: Cu, 8 mg; Fe, 0.05 g; Mn, 0.07 g; Zn, 0.05 g; I, 1.2 mg; Se, 0.2 mg.

[c] Antioxidant butylated hydroxytoluene.

[d] All diets contained: AMEn, 2,750 kcal/kg; Calcium, 30 g/kg; Non-phytate phosphorus, 4 g/kg; and Sodium, 2.50 g/kg.

Table 2
Proportions of the summit diet diluted with the corresponding nitrogen-free diet in the valine (Val), isoleucine (Ile), and tryptophan (Trp) trials and the resulting concentrations of the limiting amino acids in the diets.

Valine			Isoleucine			Tryptophan		
Summit	N-free	Val	Summit	N-free	Ile	Summit	N-free	Trp
%	%	g/kg	%	%	g/kg	%	%	g/kg
20	80	1.90	20	80	1.75	20	80	0.52
30	70	2.86	30	70	2.63	30	70	0.78
40	60	3.81	40	60	3.50	40	60	1.04
50	50	4.76	50	50	4.38	50	50	1.30
60	40	5.71	60	40	5.25	60	40	1.55
70	30	6.66	70	30	6.13	70	30	1.81
100	0	9.52	100	0	8.75	100	0	2.59
20	80	2.86[a]	20	80	2.63[b]	20	80	0.78[c]

[a] 1.016 g L-Val 96.5%/kg added to First level$_{Val}$.

[b] 0.995 g L-Ile 98.5%/kg added to First level$_{Ile}$.

[c] 0.026 g L-Trp 98%/kg added to First level$_{Trp}$.

2.5. Modelling of responses

The responses (Y) on efficiency of utilization and rate of lay (%) were regressed as a function of the amino acid intake (X) in mg/hen per day using the broken line model with one slope for rate of lay ($Y = L + U \times (R\text{-}X)$) and two slopes for efficiency of utilization ($Y = L + U \times (R\text{-}X) \times V + (X\text{-}R)$), where X is the input (amino acid intake) and Y is the output (rate of laying or efficiency of utilization) of the models, L is the maximum response of the model, R is the amino acid intake for maximum response, and the parameter U and V represents the slope in the models.

2.6. Estimating amino acid intake using a factorial model

Two models were compared, one from the literature (M1) and the other a model developed in this study (M2). The model M1 ($AAI = [(AA_m/0.85) \times BW] + [BWG \times (0.21 \times AA_t)] + [EO \times (63 \times AA_y + 158 \times AA_t)]$) was proposed by Bornstein et al. (1979), where: AAI is the amino acid intake (mg/day); AA_m is the amino acid for maintenance; 0.85 is the protein absorption rate; BW is the body weight; BWG is the body weight gain; 0.21 is the amount of protein in gain; AA_t is the amino acid in the protein fraction of the tissue; EO is the egg output; 63 is the nitrogen concentration in the egg; AA_y is the fraction of amino acid in egg yolk protein; and 158 is the amount of nitrogen in the tissue. The coefficient used for weight gain (0.21) was obtained from Bornstein et al. (1979). A second model was proposed (M2) $AAI = [AA_m \times (BW \times 0.196)^{0.73}] + [((N_{egg} \times EO) \times AA_{egg})/k]$; where AAI is the amino acid intake; AA_m is the amount of amino acid for maintenance ($mg/BP_m^{0.73} \times u$) obtained from Lima et al. (2016); *u* is the maturity rate obtained dividing body protein weight at age t (BPt) by the body protein weight at maturity (BP_m), i.e. u = BPt/BPm. In this study, *u* was considered as 1 because the broiler breeders were mature; BW is the body weight of the hens; EO is the egg output; k is the efficiency of utilization from the present data; 0.196 is the amount of nitrogen contained in the body of the birds without feathers analysed in the laboratory; N_{egg} is the amount of nitrogen in the egg (1.89 g N/100 g according to Fisher, 1998); and AA_{egg} is the amount of amino acids in the egg (mg/g N) obtained from composition presented by Lunven et al. (1973).

To predict valine, isoleucine, and tryptophan intake, the data of body weight and egg mass of 60 broiler breeder hens of Cobb (500) strain were used. These data were obtained from individual monitoring of the hens during the period from 25 to 60 weeks of age.

2.7. Statistical analysis

The broken line model with one slope and with two slopes utilized for rate of lay and efficiency of utilization was estimated using the PROC NLIN procedure. The linear plateau models were adjusted according to the procedures described by Robbins et al. (2006). The average values (x_i) obtained were standardized ($z = x_i\text{-}\mu)/\sigma$) considering μ (average) and the σ (deviation) of the egg output and body weight. Afterwards, we calculated the corresponding values in the cumulative distribution (NORM.DIST.S (*z*;true)) in Excel®. The statistical analyses were performed using SAS (2008), version.

3. Results

3.1. Responses of broiler breeder hens to dietary valine, isoleucine, and tryptophan

Bird responses to different levels of dietary Val, Ile, and Trp are shown in Table 3. The additional response (control diet) seen with the supplementation of the synthetic amino acid confirmed that Val, Ile, and Trp were the first limiting amino acid.

3.2. Feed intake

Each hen received 150 g of feed per day, however birds fed with lowest level and second-lowest level of the amino acid diets had lower feed intake in comparison to other treatments for the three amino acids assessed (Table 3).

3.3. Rate of lay, egg weight, and egg output

The highest rates of lay were observed for birds fed the 0.952 g/kg of the valine diet, reaching 69% for Val and 69% for Ile. Regarding Trp, birds on the 1.81 g/kg diet had the best rate of lay (67%). On the other hand, hens feeding on the lowest amino-acid diet produced 44%, 40%, and 42% fewer eggs than the maximally performing diets for Val, Ile, and Trp, respectively. Birds fed lowest level or second-lowest level of the amino acid tended to lay eggs that were approximately 10% lighter than those from birds feeding on sixth or seventh level of the amino acid for the three amino acids evaluated. Egg output was also reduced by 61%, 43%, and 42% for birds feeding on lowest level when compared to the maximally performing diets for Val, Ile, and Trp, respectively. Output was especially affected with the Val-lowest level diet (18.6 g/hen per day).

3.4. Modelling the responses: amino acid intake vs. rate of lay and efficiency of utilization

Adjusted models for rate of lay in function of dietary amino acid intake were:

Table 3

Average responses to treatments and standard deviation (± SD) for daily feed intake (g/hen), daily amino acid intake (mg/hen), daily rate of lay (%), egg weight (g), daily egg output (g/bird), body weight (kg), and efficiency of broiler breeder hens from 53 to 57 weeks of age.

Levels g/kg	Feed Intake	Amino acid intake	Rate of lay	Egg weight	Egg output	Body weight	Efficiency
Valine							
1.90	119.8	227.7	38.9	62.4	18.6	4.1	152.0
2.86	136.3	389.7	53.1	62.2	32.1	4.2	117.9
3.81	144.9	552.1	65.2	66.3	44.3	4.5	103.6
4.76	141.9	675.6	63.9	69.5	45.7	4.4	80.3
5.71	143.9	822.2	62.6	68.9	44.8	4.5	57.5
6.66	147.5	982.1	68.3	70.7	47.2	4.7	48.9
9.52	146.9	1398.2	69.3	69.3	45.1	4.7	29.8
2.86[a]	132.9	380.3	50.2	67.7	28.8	4.0	97.1
Probability	0.0006	< 0.0001	< 0.0001	0.1016	< 0.0001	0.0024	< 0.0001
n	61	61	61	56	57	61	52
Isoleucine							
1.75	131.4	230.0	41.8	65.1	27.9	4.0	145.0
2.63	141.8	373.0	56.4	68.9	39.8	4.3	93.3
3.50	144.5	505.7	65.2	67.0	43.6	4.3	68.2
4.38	141.3	618.7	60.2	70.8	42.8	4.6	55.6
5.25	146.2	767.4	60.7	72.4	43.9	4.7	42.5
6.13	146.1	895.3	59.5	67.1	41.1	4.6	32.6
8.75	147.3	1289.2	69.2	70.6	49.0	4.6	24.3
2.63[b]	140.1	368.4	45.9	67.1	30.9	4.2	73.3
Probability	0.0017	< 0.0001	< 0.0001	0.1391	< 0.0001	0.0017	< 0.0001
n	62	62	59	62	59	62	50
Tryptophan							
0.52	135.1	70.3	38.8	65.7	26.0	3.9	120.28
0.78	139.8	109.0	52.0	65.9	31.9	4.4	103.4
1.04	139.9	145.5	57.6	70.9	40.6	4.3	73.8
1.30	145.0	188.5	52.7	72.2	43.2	4.4	59.3
1.55	143.6	222.6	53.1	71.7	43.3	4.6	47.9
1.81	147.1	266.2	66.5	70.1	44.9	4.6	42.1
2.59	148.1	383.6	63.8	72.9	44.9	4.9	28.0
0.78[c]	132.6	98.7	40.2	66.3	27.9	3.9	77.7
Probability	0.0005	< 0.0001	0.0020	0.0345	< 0.0001	< 0.0001	< 0.0001
n	64	64	62	62	58	64	53

[a,b,c]Control diet.

n: number of observations.

[a] Added 1.016 g/kg of L-valine.

[b] Added 0.995 g/kg of L-isoleucine.

[c] Added 0.026 g/kg L-Tryptophan.

$$\text{Rate of lay}_{\text{Val}} = 67.19\text{-}0.08 \times (596.7\text{-X});\ \text{if X} > 596.7 \text{ then } (596.7\text{-X}) = 0 \tag{1}$$

$$\text{Rate of lay}_{\text{Ile}} = 66.31\text{-}0.09 \times (499.3\text{-X});\ \text{if X} > 499.3 \text{ then } (499.3\text{-X}) = 0 \tag{2}$$

$$\text{Rate of lay}_{\text{Trp}} = 64.22\text{-}0.22 \times (167.5\text{-X});\ \text{if X} > 167.5 \text{ then } (167.5\text{-X}) = 0 \tag{3}$$

Errors associated with L, U, and R were, respectively: 3%, 24%, and 10% for Val; 3%, 20%, and 8% for Iso; and 3%, 18%, and 6% for Trp.

Adjusted models for efficiency of utilization in function of dietary amino acid intake were:

$$\text{Efficiency}_{\text{Val}} = 70.52\text{-}0.05 \times (\text{X-}597.8) + 0.35 \times (597.8\text{-X});\ \text{if X} < 597.8 \text{ then } (\text{X-}597.8) = 0 \text{ and if X} > 597.8 \text{ then } (654\text{-X}) = 0 \tag{4}$$

$$\text{Efficiency}_{\text{Ile}} = 58.70\text{-}0.04 \times (\text{X-}499.3) + 0.31 \times (499.3\text{-X});\ \text{if X} < 499.3\text{, then } (\text{X-}499.3) = 0 \text{ and if X} > 499.3\text{, then } (499.3\text{-X}) = 0 \tag{5}$$

$$\text{Efficiency}_{\text{Trp}} = 55.46\text{-}0.13 \times (\text{X-}167.5) + 0.72 \times (167.5\text{-X});\ \text{if X} < 167.5\text{, then } (\text{X-}167.5) = 0 \text{ and if X} > 167.5\text{, then } (167.5\text{-X}) = 0 \tag{6}$$

Errors associated with L, U, R, and V were, respectively: 9%, 21%, 5%, and 10% for Val; 4%, 12%, 8%, and 6% for Ile; and 8%, 23%, 6%, and 11% for Trp.

Rate of lay increased with increasing amino acid intake up to the response plateau and efficiency of utilization decreased with

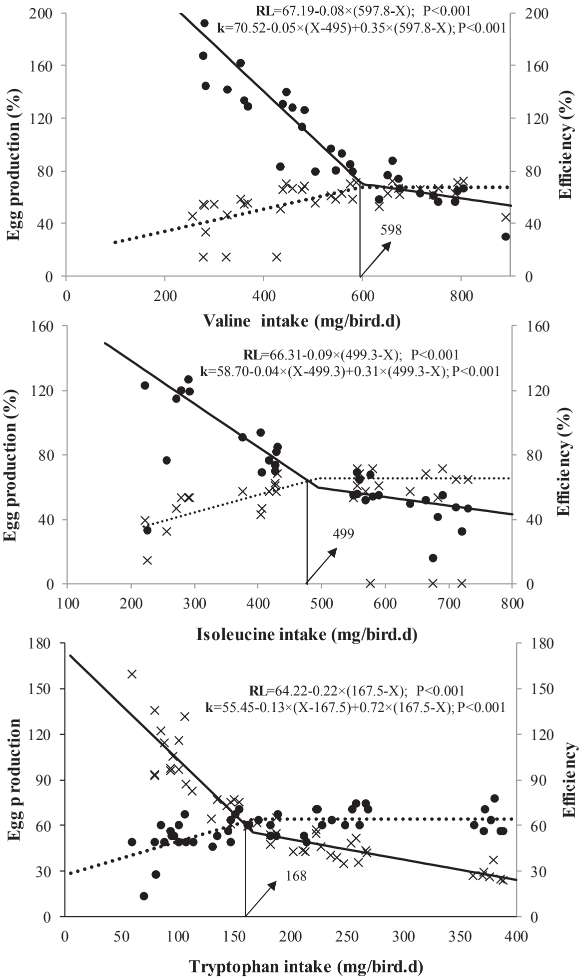

Fig. 1. Relation between amino acid intakes and Rate of lay, RL (……, ×), and efficiency, k (▬▬, ●).

increasing amino acid intake (Fig. 1). The adjusted model for utilization efficiency includes two slopes (Eqs. (4)–(6)). The amino acid concentration at which the second slope starts is the same at which the rate of lay reaches the response plateau (Eqs. (1)–(3)).

3.5. *Factorial model for the estimation of ideal valine, isoleucine, and tryptophan intake*

Ideal amino acid intake is given by the factorial model:

$$AAI = [AA_m \times (BW \times 0.196)^{0.73}] + [((N_{egg} \times EO) \times AA_{egg})/k] \tag{M2}$$

Where: AAI = amino acid intake; AA_m = maintenance amino acid intake; BW = body weight measured as $mg/BP_m^{0.73} \times u$ where u = proteic weight in time/proteic weight at maturity, a ratio that equals 1 for mature animals; 0.196 = amount of nitrogen contained in the body of the birds without feathers; $N_{egg,}$ = amount of nitrogen in the egg (1.89 g/100 g) (Fisher, 1998); EO = egg output (g); AA_{egg} = amount of amino acids in the egg (mg/g N) using values for each amino acid reported by Lunven et al. (1973); and k is the efficiency of utilization determined in the present study. Equations obtained for the three amino acids were:

$$AAI_{Val} = [247 \times (BW \times 0.196)^{0.73}] + [((1.89 \times EO) \times 413)/0.705] \tag{7}$$

$$AAI_{Ile} = [134 \times (BW \times 0.196)^{0.73}] + [((1.89 \times EO) \times 338)/0.587] \tag{8}$$

$$AAI_{Trp} = [37 \times (BW \times 0.196)^{0.73}] + [((1.89 \times EO) \times 108)/0.554] \tag{9}$$

Table 4
Prediction of valine, isoleucine, and tryptophan intake for 60 broiler breeder hens from 25 to 60 weeks of age using two mathematic models with body weight (BW), body weight gain (BWG), and egg output (EO) data.

Age weeks	BW Kg	BWG g/day	EO g/day	Valine intake		Isoleucine intake		Tryptophan intake	
				mg/day					
				M1[a]	M2[b]	M1[a]	M2[b]	M1[a]	M2[b]
25	3.89	6	37.1	790	610	601	518	111	168
30	4.10	6	53.9	1057	803	793	708	149	232
35	4.32	6	55.7	1098	831	824	732	155	240
40	4.54	4	55.6	1108	838	834	735	156	240
45	4.68	4	50.0	1032	782	781	677	145	220
50	4.82	0	48.7	1020	772	774	665	143	216
55	4.83	0	47.7	1005	761	764	654	141	212
60	4.83	0	45.6	974	738	741	631	137	205

[a] M1. $AAI = [(AA_m/0.85) \times BW] + [BWG \times (0.21 \times AA_t)] + [EO \times (63 \times AAy + 158 \times AA_t)]$ from Hurwitz and Bornstein (1973).

[b] M2. $AAI = [AA_m \times (BW \times 0.196)^{0.73}] + [((N_{egg} \times EO) \times AA_{egg})/k]$ developed in this study.

3.6. Model simulation and evaluation

Factorial models M1 and M2 were simulated with body weight and egg output from 60 individually monitored hens. Simulation results are shown in Table 4. The M1 model compared to M2 predicted 24% and 13% higher intakes for Val and Ile, respectively. The predicted intake for Trp was 52% lower in M1 than in M2. The observed differences between the models remained during the whole period assessed.

3.7. Application of the factorial model to estimate amino acid intakes for a population

Average egg output and body weight values for 60 hens at 25, 30, 35, 40, 45, 50, 55, and 60 weeks of age were applied in the M2 model to predict Val, Ile, and Trp intake (Table 5).

Table 5
Population data for 60 broiler breeder hens and prediction of the minimum (min), average (μ), and maximum (max) values for valine, isoleucine, and tryptophan intake based on the application of the factorial models.

Age weeks	Population data					
	Egg outptut (g)			Body weight (kg)		
	Min	μ ± SD	max	min	μ ± SD	max
25	13	37 ± 14	53	3.3	3.9 ± 0.3	4.4
30	20	54 ± 10	70	3.5	4.1 ± 0.3	4.6
35	35	56 ± 7	70	3.7	4.3 ± 0.4	5.0
40	29	56 ± 10	80	3.6	4.5 ± 0.4	5.3
45	19	50 ± 10	65	3.6	4.7 ± 0.4	5.5
50	15	49 ± 10	68	3.4	4.8 ± 0.4	5.5
55	24	48 ± 9	68	3.4	4.8 ± 0.4	5.6
60	22	46 ± 10	63	3.1	4.8 ± 0.5	5.7

	Amino acid intakes predicted								
	Valine			Isoleucine			Tryptophan		
	Min	μ ± SD	max	min	μ ± SD	max	min	μ ± SD	max
	mg/bird per day								
25	337	610 ± 157	784	247	518 ± 157	691	77	168 ± 53	226
30	436	803 ± 112	987	338	708 ± 112	889	107	232 ± 38	293
35	608	831 ± 80	989	509	732 ± 80	890	164	240 ± 27	293
40	555	838 ± 108	1112	448	735 ± 108	1006	143	240 ± 37	331
45	441	782 ± 112	961	334	677 ± 112	851	105	220 ± 38	279
50	409	772 ± 117	1001	297	665 ± 116	885	92	216 ± 39	290
55	511	761 ± 104	998	399	654 ± 103	886	126	212 ± 35	291
60	465	738 ± 117	953	364	631 ± 116	834	115	205 ± 39	273

SD = standard deviation.

For egg output, the coefficient of variation (CV) at 25 weeks was high (35%) because of the low rate of lay, but after this time point it stabilized at the average value of 19%. For body weight, the CV ranged between 7 and 10% of the average value of 4.5 kg.

The average egg output values and standard deviation accounted for 46.55% of the population, whereas maximum egg output values accounted for 90.07% of the population. For body weight, average values corresponded to 54.55% of the population, and maximum values to 94.66%.

Predicted values for Val, Ile, and Trp intake varied by 503, 500, and 168 mg/hen per day, respectively (Table 5). Differences between the maximum and average intakes were 206 (Val), 202 (Ile), and 68 (Trp) mg/hen per day, yielding maximum values approximately 29% higher than average intake values. Differences between the average and minimum intakes were 297 (Val), 298 (Ile), and 100 (Trp) mg/hen per day, corresponding to a difference of approximately 43%.

4. Discussion

We evaluated the responses of broiler breeder hens to different intakes of Val, Ile, and Trp intake. Furthermore, we determined amino acid efficiency of utilization, and adapted the mathematical model originally developed by Bornstein et al. (1979). Our results include the dose-responses obtained by progressive dilution of the three amino acids in breeder diets. However, more importantly, we provide a revised mathematical model that can be used to define amino acid levels depending on producer goals and needs.

4.1. Responses of broiler breeder hens to dietary valine, isoleucine, and tryptophan

We applied the concept of relative deficiency by dilution technique (Fisher and Morris, 1970) to limit the amount of Val, Ile, and Trp amino acids in dietary protein. Compared to lowest level, the control diet for each amino acid increased rate of lay, egg weight, and egg output, demonstrating that the amino acids were in fact limiting (Table 3). The control diet for Val provided the highest improvement in rate of lay compared to lowest level, followed by Ile and Trp, indicating the limiting potential of these amino acids. Previous studies using the same methodology also based their conclusions about limiting potential on the relative responses to their highest dilution and control diets (Wethli and Morris, 1978; Bowmaker and Gous, 1991).

Dietary amino acid concentration had little influence on feed intake (Table 3). Only the lower amino acid intake resulted in decreased intake. Another study reported a similar effect (Gous et al., 1987). This counter-intuitive effect may result from the larger volumes as well as higher relative concentration of other nutrients in the more diluted diets. Among amino acids, feed intake only stabilized with diet 1.30 g/kg for Trp and third level of the amino acid for the other two amino acids. The Trp has been shown to increase feed intake in other farm animals (Sève, 1999). Thus, the observed difference may result from a depressant effect on feed intake of very low Trp concentrations. Similar Trp effects on hens have been previously reported (Morris and Wethli, 1978).

Egg weight and rate of lay decreased with lower amino acid concentrations (Table 3). Previous studies evaluating other amino acids have reported two-fold greater reductions (Morris and Gous, 1988; Bowmaker and Gous, 1991). This discrepancy may result from the age of hens. The older hens used in the current study have larger body reserves and more easily mobilize tissue mass for egg formation, even at very low amino acid concentrations (Bowmaker and Gous, 1991).

4.2. Utilization efficiency and rate of lay

At lower amino acids concentrations, hens reduced protein intake to near-maintenance requirements, when they would be expected to stop laying eggs. However, in parallel with reducing body weight (data not shown), broiler breeders kept producing more than 2 eggs per week, totalling 18 to 27 eggs during the entire experimental period. These results agree with those previously obtained by Bowmaker and Gous (1991) who reported the production of one egg per week in parallel with body weight loss. Thus, broiler breeders react differently to dietary restriction when compared to laying hens. The former probably prioritize egg laying even at the expense of body energy stores.

Efficiency of utilization increased in lower amino acid concentrations, probably because, with the mobilization of body mass towards egg laying, the apparent feed conversion improved. The amino acid concentration at which the second slope of the efficiency of utilization curve starts is the same at which the rate of lay reaches the response plateau for the three amino acids tested (Fig. 1). These results agree with the hypothesis that broiler breeder efficiency is a complex variable related to rate of lay (Fisher, 1994, 1998). Fisher et al. (2001) used a methodology similar to ours to calculate efficiency of utilization of broiler breeder hens of four different ages. The authors found that lysine efficiency of utilization was 82%, 76%, 59%, and stabilized at 57% for hens aged 26, 37, 48, and 60 weeks, respectively. Similar lysine efficiencies were reported in other studies (Bowmaker and Gous, 1991; Silva et al., 2015a). We report here a compatible average efficiency for the three amino acids (Val, Ile, and Trp) of 62%.

4.3. Factorial model for the estimation of ideal valine, isoleucine, and tryptophan intake

Based on maintenance and efficiency of utilization coefficients, as well as physiological aspects, we revised the amino acid requirement model originally proposed by Hurwitz and Bornstein (1973). The main alterations we propose to the model involve metric, units, and maintenance percentage values. The maintenance requirement coefficient we propose includes protein weight at maturity expressed as $\mathrm{mg}/_{\mathrm{kg}}\mathrm{BP_m}^{0.73} \times \mathrm{u}$ as previously recommended by Emmans (1989) in substitution for body weight. This change accounts for widely different body fat percentages, which may distort results because lipid reserve maintenance does not require amino acids. We also considered 100% efficiency for maintenance as opposed to the 85% proposed by Bornstein et al. (1979). Other models also employ 100% efficiency for maintenance (Martin et al., 1994; Samadi and Liebert, 2007a, 2007b, 2008). This change is justified by the very low amino acid concentrations required for maintenance. The efficiency of utilization value introduced in the model was 60% for all ages, as opposed to the 85% previously used (Hurwitz and Bornstein, 1973). We propose this change based on the fact that broiler breeders require higher intake than laying hens to deposit the same amount of egg amino acids (Silva et al., 2015a,b). However, because this input value is greatly related to rate of lay (Fisher, 1994, 1998), further studies can improve on it by modelling efficiency of utilization in a dynamic form.

After the changes introduced, the M2 model predicted lower required intake of Val and Ile. This results mostly from the correction in maintenance efficiency. On the other hand, our M2 model yielded higher requirements of Trp because of new amino acid deposition coefficients that reflect ideal egg amino acid concentrations in the egg (Lunven et al., 1973).

We measured the egg output and body weight of 60 individual hens to evaluate the predictive power of the model at minimum, average, and high performances (Table 5). This was done according to previous suggestions that limiting amino acid intakes should be calculated based on population variations (Fisher et al., 1973) Depending on the distribution of high and low-productivity birds, the average population will not represent 50% of individuals. Here, we found that recommended intake would be optimal for 47% of the population instead of 50%, because some individuals were producing with long pauses between laying sequences, also justifying the high CV for the minimum predicted intake of Val, Trp, and Ile.

The difference between the maximum and average intake corresponded to 30%. This value can be used as a correlation between population average and the average maximum value. Thus, the average value times 1.3 approximates the maximum intake for 90% of this population of individuals. However, this value varies depending on the distribution and uniformity of body weight and egg output. It is appropriate to consider economic aspects of the determination of the limiting amino acid intake (Fisher et al., 1973).

The M2 predicted intake was 25% and 29% higher for Val and Trp, respectively, and 12% lower for Ile than recommended by the NRC (1994), considering the period of 25 to 60 weeks of age. A lower intake of Ile has also been recommended by Fisher (1998). Ekmay et al. (2013) found that rates of lay for Val, Ile, and Trp reach a plateau at 829, 794, and 234 mg/d, respectively. These authors do not provide intake variation in the estimates, but based on the values provided, the M2 model differed by −5, −69, and 3.3 mg/d of Val, Ile, and Trp, respectively, in the same experimental period.

The M2 model yielded lower Ile intakes than recommended by Ekmay et al. (2013) at all ages. However, our model only takes into account of body protein weight and egg amino acids to predict intake, with no fertility variable. However, the best fertility rates observed by Ekmay et al. (2013) were at Ile intakes lower than 625 mg/d. Their average optimal intakes for rate of lay and fertility would be 709.5 mg/d and 15.5 mg/d above the value predicted by our model.

Altogether, our results suggest that the M2 model incorporates the most physiologically relevant units, and values to provide accurate estimates of amino acid requirements by broiler breeder hens. Future work should refine M2 taking into account the dynamics of utilization efficiency, and considering an association between efficiency of utilization and fertility.

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