Digestible threonine for slow-growing broilers: performance, carcass characteristics, intestinal mucin, and duodenal morphometry

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ABSTRACT - Five experiments were conducted to estimate the digestible threonine requirements of slow-growing broilers: experiment 1, starter phase (days 10 to 28); experiment 2, grower I phase (days 29 to 49); experiment 3, grower II phase (days 50 to 69); experiment 4, finisher phase (days 70 to 84); and experiment 5, which was specifically conducted to determine the production of intestinal mucin over two periods (days 50 to 69 and 70 to 84). Different birds were used in all experiments. A completely randomized design with five treatments and four replicates was used. Treatments consisted of increasing the values of digestible threonine in the diet through basal feed supplementation with L-threonine (98.5%), which was added instead of cornstarch. The following values of digestible threonine were investigated: 0.622, 0.697, 0.772, 0.847, and 0.922% in experiment 1; 0.586, 0.662, 0.738, 0.814, and 0.890% in experiment 2; 0.570, 0.640, 0.71, 0.780, and 0.850% in experiments 3 and 5; and 0.520, 0.595, 0.670, 0.745, and 0.820% in experiments 4 and 5. The digestible threonine values presented quadratic effects on feed conversion ratio in experiments 1, 2, and 3. Digestible threonine values of 0.628 and 0.609% resulted in higher villus height and greater duodenal crypt depth, respectively. Digestible threonine values of 0.762, 0.767, and 0.733% may be recommended for the starter, grower I, and grower II phases, respectively, based on the best feed conversion ratio. In addition, a digestible threonine level of 0.694% may be recommended for the finisher phase based on the highest production of intestinal mucin.

Key Words: amino acid, nutritional requirements, villus

Introduction

Threonine is considered the third limiting amino acid for broilers fed corn-soybean meal-based diets (Ayasan and Okan, 2006), being an essential amino acid for broilers, and has key functions in the animal body. It is the main component of intestinal mucus, which aids in nutrient digestion and absorption and consists primarily of mucin produced by goblet cells (Smirnov et al., 2006). Threonine may represent up to 40% of the amino acids that make up these glycoproteins (Abassi et al., 2014).

Decreased intestinal mucin production can reduce nutrient utilization by minimizing the intestinal mucus layer (Horn et al., 2009).

Threonine also affects animal immune systems (Li et al., 2007) and may account for 7 to 11% of all amino acids in immunoglobulins (Sandberg et al., 2007). It participates in the uric acid formation and in protein synthesis (Eftekhari et al., 2015). Deficiencies in digestible threonine may lead to impaired function of the aforementioned organic molecules.

Regarding the feed nutritional values, there is no difference in preceacal amino acid digestibility between fast- and slow-growing broiler strains in soybean cake; thus, the results estimated could be applied to both types of broiler strains (Ganzer et al., 2017).

However, the nutritional requirements may vary according to the growth potential. Most research assessing the nutrient requirements have been conducted using fast-growing broilers. Ayaşan et al. (2009) concluded that the

0.75% level of threonine maximizes body weight gain in the period from 22 to 42 days of age. Little information is known on the threonine requirements of slow-growing broilers. The CobbSasso150 (Cobb-Vantress, 2008) slow-growing broiler strain manual recommends total threonine values of 0.90, 0.80, and 0.76% for the starter, grower, and finisher phases, respectively.

Redbro Plumé is a strain of French origin and reaches an average final weight of 2200 g between 70 and 80 days, consuming between 5700 and 6300 g of ration; it has feathered neck, feathers of strong red colour, and black tail (Miranda et al., 2005).

This study aimed to assess the effects of digestible threonine on performance and carcass traits of slow-growing broilers during the starter (days 10 to 28), grower I (days 29 to 49), grower II (days 50 to 69), and finisher (days 70 to 84) phases; duodenal morphometry in the finisher phase and production of intestinal mucin in the starter, grower II, and finisher phases were assessed.

Material and Methods

Research on animals was conducted according to the institutional committee on animal use (case no. 23083.011134/2014-02). The experiments were performed in Seropédica, Rio de Janeiro, Brazil (latitude 22º45' S and longitude 43º41' W).

A total of 1600 Redbro Plumé male broiler chicks vaccinated against Marek's, Newcastle, and Gumboro diseases were initially purchased for the experiments and housed in a conventional broiler house. The birds remained in the broiler house until they were transported for the experiments and received water and food *ad libitum*.

Five experiments were conducted from June 29 to September 21, 2015. In experiment 1 (days 10 to 28), 200 broiler chickens with a uniform mean weight were selected and housed in metabolic cages (measuring 0.90 × 0.85 × 0.40 m) to evaluate broiler performance and mucus production in response to increasing levels of digestible threonine. Under each cage, trays covered with plastic were placed to collect excreta and determine intestinal mucin production.

Experiments 2 (grower phase I, from days 29 to 49), 3 (grower phase II, from days 50 to 69), and 4 (finisher phase, from days 70 to 84) were conducted to investigate response of performance and carcass characteristics of broilers fed increasing proportions of digestible threonine. The experimental pens in which the birds were housed measured 4 × 5 m and contained wood shavings beds, feeders, and water troughs.

Experiment 5 was conducted with different broilers housed during two different age periods (days 50 to 69 and 70 to 84). Initially, 60 broilers of uniform mean weight were selected at 50 days of age and housed in metabolic cages in a similar manner to that mentioned for experiment 1 for total excreta collections and to determine intestinal mucus production. At the end of the experimental period, at 69 days of age, the broilers were removed from the cages, and the cages were sanitized. Then, an additional 60 broilers at 70 days of age were housed in the cages and remained in these locations until 84 days of age to allow for another round of excreta collection and determination of intestinal mucus. Outside the experimental periods, birds were fed reference feeds according to the recommendations of the Brazilian table for poultry and swine (Rostagno et al., 2011).

The lighting programme followed the recommendations of the Normative Instruction Number 46 of the Ministry of Agriculture, Livestock and Food Supply and consisted of 16 h of light and 8 h of dark (Brasil, 2011).

The experiments were conducted in a completely randomized design with five treatments and four replicates. Each replicate consisted of 10 chicks in experiment 1, 22 broilers in experiments 2 and 3, 20 broilers in experiment 4, and three broilers in experiment 5 (for each period studied). A total of 200, 440, 440, 400, and 120 birds were used in experiments 1, 2, 3, 4, and 5, respectively.

The experimental diets contained increasing proportions of digestible threonine and were obtained by supplementing the basal feed with L-threonine (98.5%), which was added instead of cornstarch. The treatments were as follows: 0.622, 0.697, 0.772, 0.847, and 0.922% digestible threonine in experiment 1; 0.586, 0.662, 0.738, 0.814, and 0.890% digestible threonine in experiment 2; 0.570, 0.640, 0.710, 0.780, and 0.850% digestible threonine in experiment 3; and 0.520, 0.595, 0.670, 0.745, and 0.820% digestible threonine in experiment 4. The treatments studied in experiment 5 were the same experimental diets as in experiment 3 (for grower phase II) and experiment 4 (for the finisher phase) (Table 1).

In all experiments, the basal feed formula was based on corn and soybean meal and met the requirements mentioned by Rostagno et al. (2011). The criterion for determining the feed requirement was the mean body weight of the animals rather than ages as described in the Brazilian table for poultry and swine (Rostagno et al., 2011). The values of available phosphorus and calcium suggested by Pinheiro et al. (2011a, 2011b) were used.

In experiments 1-4, body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), threonine intake

(TI), and threonine utilisation efficiency (TUE) were assessed. Threonine intake was calculated by multiplying the mean FI by threonine digestibility, and TUE was assessed by dividing the mean BWG by TI.

At the end of experiments 3, 4, and 5, four broilers per replicate were selected based on the representative mean weight of the replicates; these animals were slaughtered by cervical dislocation, bled, scalded, plucked, and eviscerated. They were then subjected to a pre-cooling process with a 5-min dripping step. The carcasses were weighed and

Table 1 - Ingredient and nutrient compositions of basal diets used in experiments

Iem	Experiment			
	1	2	3 and 5	4 and 5
Ingredient (g/kg as fed)				
Corn grain (7.73% CP)[1]	658.36	695.35	709.58	749.18
Soybean meal (46.26% CP)[1]	277.74	251.42	236.55	198.58
Soybean oil	8.57	7.07	13.65	10.33
Dicalcium phosphate	15.86	10.233	12.45	12.77
Corn starch	6.00	7.00	7.00	7.00
Calcitic limestone	18.69	8.42	8.43	8.47
Common salt	4.81	4.56	4.43	4.37
DL-methionine	3.31	2.87	2.40	2.81
L-lysine HCl	4.11	8.38	3.01	3.84
Vitamin mixture[2]	1.00	1.00	1.00	1.00
Mineral mixture[3]	1.00	1.00	1.00	1.00
Choline chloride	0.55	0.50	0.40	0.40
Butylated hydroxytoluene	-	0.10	0.10	0.10
L-tryptophan	-	-	-	0.15
Nutritional composition (%)[4]				
Linoleic acid	1.955	1.923	2.283	2.150
Calcium[5]	1.160	0.680	0.680	0.680
Chlorine	0.340	0.326	0.318	0.315
Metabolizable energy (kcal/kg)	2980	3050	3100	3125
Available phosphorus[6]	0.394	0.325	0.325	0.325
Digestible leucine	1.489	1.437	1.402	1.315
Total lysine[7]	1.283	1.540	1.084	1.049
Digestible lysine	1.170	1.442	0.987	0.960
Digestible methionine and cysteine	0.835	0.773	0.713	0.722
Digestible methionine	0.582	0.529	0.477	0.502
Potassium	0.699	0.661	0.638	0.580
Crude protein	18.58	18.00	16.93	15.60
Sodium	0.210	0.200	0.195	0.192
Total threonine[7]	0.723	0.686	0.655	0.608
Digestible threonine	0.622	0.590	0.570	0.520
Digestible tryptophan	0.194	0.180	0.174	0.167

CP - crude protein.
[1] Value determined in the food science laboratory of the Animal Science Institute of the Universidade Federal Rural do Rio de Janeiro.
[2] Guaranteed analysis (per kg of product): vitamin A, 6,000,000 IU; vitamin D_3, 2,000,000 IU; vitamin E, 12,000 mg; vitamin K_3, 800 mg; vitamin B_1, 1,000 mg; vitamin B_2, 4,500 mg; vitamin B_6, 1,500 mg; vitamin B_{12}, 12,000 mg; niacin, 30,000 mg; calcium pantothenate, 10,000 mg; folic acid, 550 mg; biotin, 50 g; antioxidant, 5,000 mg; excipient q.s., 1,000 g.
[3] Guaranteed analysis (per kg of product): iron (ferrous sulphate), 60,000 mg; copper (copper sulphate), 13,000 mg; manganese (manganese sulphate), 120,000 mg; zinc (zinc oxide), 100,000 mg; iodine (calcium iodine), 2,500 mg; selenium (sodium selenite), 500 mg; excipient q.s., 1,000 g.
[4] Brazilian tables for poultry and swine. The amino acids are expressed as standardized digestibility.
[5,6] Pinheiro et al. (20011a, 2011b).
[7] Analyzed amino acid.

separated into breast, wing, thigh, drumstick, and back sections, which were weighed to assess the yield relative to cold carcass weight.

At the slaughter for experiment 4, two broiler chickens were collected per replicate, and the small intestines were reserved for analysis of duodenal morphometry. After the intestines were sanitized, two samples measuring approximately 5 cm were collected from the duodenal portion and fixed in formalin solution (5%). The samples were dehydrated, deparaffinized in xylene, impregnated with resin, sliced to a thickness of 5 μm on a microtome, and mounted on slides. The slides were then stained with haematoxylin and eosin and covered with glass cover slips to permit microscopic analysis of villus height and crypt depth. A binocular microscope was used to capture the images and perform the measurements; 30 measurements per replicate were obtained. Villus height was measured from the basal area, which coincided with the upper portion of the crypt, to the apex; the crypts were measured from the base to the crypt, which indicated a villus transition area. Measurements were expressed in μm.

Quantification of crude intestinal mucin production was performed by analysis of excreta from the birds, in experiments 1 and 5. Excreta was collected twice daily (8.00 and 16.00 h) during the last three days in each experimental period. The method proposed by Horn et al. (2009) was used to perform the analysis of crude mucin. For each sample, 3 g of excreta was placed in a plastic centrifuge tube, and 20 mL of chilled (4 ºC) NaCl solution (0.15 M NaCl, 0.02 M NaN_3) were added. The solution was homogenized for 30 s and centrifuged at 12,000 g for 20 min at 4 ºC. The soluble supernatant was decanted into a pre-weighed 50-mL tube. Then, 15 mL of absolute ethanol solution was added to the supernatant, and the mucin proteins were extracted overnight at −20 ºC. The mixture was centrifuged for 10 min at 4 ºC, and the sediment retained the mucin. The pelleted mucin was washed in a mixture of 10 mL of NaCl (0.15 M NaCl, 0.02 M NaN_3) and 15 mL of absolute ethanol overnight at −20 ºC. The mixture was then centrifuged, and the sediment was washed until a clear supernatant was obtained. The supernatant was removed by aspiration, and the pellet was weighed to assess the crude mucin yield.

The data were analyzed using the statistical program SISVAR (Analysis System of Variance) (Ferreira, 2011), considering a P-value of 0.05 to be significant. Experimental responses to the values of digestible threonine were studied by regression analysis, and estimates of nutritional requirements were established, when possible, by studying the quadratic model.

Results

Digestible threonine values exerted quadratic effects on BWG (P = 0.001) and FCR (P = 0.021) and linear effects on FI (P = 0.042) and TI and TUE (P = 0.001) (Table 2). Increased BWG, which corresponded to a maximum gain estimate of 671.26 g, was estimated to require 0.716% digestible threonine. The required estimate for FCR was 0.762%, with an intake corresponding to a digestible threonine:lysine ratio of 65.12%.

Table 2 - Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), threonine intake (TI), and threonine utilisation efficiency (TUE) of slow-growing broilers in experiments 1, 2, 3, and 4

Digestible threonine (%)	BWG (g)	FI (g)	FCR	TI (g)	TUE (%)
Experiment 1 (days 10 to 28)					
0.622	655.7	1094	1.67	6.805	96.35
0.697	681.0	1098	1.61	7.653	88.98
0.772	671.7	1055	1.57	8.145	82.47
0.847	636.8	1061	1.67	8.987	70.86
0.922	631.3	1051	1.66	9.690	65.15
CV	1.56	3.28	3.13	3.54	2.89
Source of variation			Probability		
Linear regression	0.010	0.042	0.582	0.001	0.001
Quadratic regression	0.001	0.754	0.021	0.915	0.657
Experiment 2 (days 29 to 49)					
0.586	1160	3044	2.62	17.84	65.02
0.662	1129	2757	2.44	18.25	61.86
0.738	1173	2679	2.28	19.77	59.33
0.814	1162	2698	2.32	21.96	52.91
0.890	1147	2818	2.46	25.08	45.73
CV	2.78	4.08	3.62	7.90	9.62
Source of variation			Probability		
Linear regression	0.898	0.130	0.050	0.001	0.001
Quadratic regression	0.718	0.001	0.002	0.815	0.676
Experiment 3 (days 50 to 69)					
0.570	1288	4300	3.34	24.51	52.55
0.640	1327	4277	3.22	27.37	48.48
0.710	1391	4202	3.02	29.83	46.63
0.780	1368	4070	2.97	31.75	43.09
0.850	1299	4214	3.24	35.82	36.26
CV	2.62	1.27	2.76	1.46	2.88
Source of variation			Probability		
Linear regression	0.275	0.080	0.005	0.0001	0.0001
Quadratic regression	0.0001	0.015	0.0001	0.380	0.110
Experiment 4 (days 70 to 84)					
0.520	965.5	3079	3.19	16.01	60.31
0.595	1009	2964	2.94	17.63	57.23
0.670	997.6	3195	3.20	21.41	46.59
0.745	991.0	3111	3.14	23.18	42.75
0.820	951.0	3165	3.33	25.95	36.65
CV	10.36	3.61	9.95	3.67	9.95
Source of variation			Probability		
Linear regression	0.780	0.323	0.095	0.0001	0.0001
Quadratic regression	0.407	0.403	0.920	0.841	0.941

CV - coefficient of variation.

Threonine had a significant quadratic effect (P = 0.001) on mucin production, with a 0.775% requirement for maximum mucin production, which corresponded to an intestinal mucin production of 524.1 g/kg excreta (Table 3).

We verified quadratic effects for FI (P = 0.001) and FCR (P = 0.002), but linear effects were observed for TI (P = 0.001) and TUE (P = 0.001) (Table 2). A 0.767% requirement was estimated for the minimum FI and FCR according to the quadratic model. The dietary level of digestible threonine had no effect on cut yields except for the back, which showed a quadratic effect (P = 0.030) with an estimated requirement of 0.738% digestible threonine (Table 4).

In experiment 3, BWG (P = 0.0001), FI (P = 0.015), and FCR (P = 0.0001) were significantly affected by the dietary threonine level (Table 2). The requirement for digestible threonine estimated for BWG was 0.720%, which corresponded to a gain of approximately 1378 g during the experimental period. Conversely, the requirement for digestible threonine estimated for the lowest FI was 0.777%. The requirement for FCR was estimated at 0.733%. Yields of breast, thigh and drumstick, and wing showed linear effects (P = 0.001). The negative linear effect on breast yield showed that the yield of that cut decreased with increasing dietary values of digestible threonine (Table 4).

No digestible threonine effects on BWG, FI, or FCR (P>0.05) were observed (Table 2). Threonine values affected only thigh and drumstick yields (P = 0.022), which showed a linear effect (Table 4).

Villus height (P = 0.001), crypt depth (P = 0.002), and villus height:crypt depth ratio (P = 0.001) were affected by the digestible threonine values tested (Table 5). The quadratic effect observed in the duodenal morphometry study of villus height, crypt depth, and duodenal villus height:crypt depth ratio yielded an estimation of the requirement for the highest villus height of 0.628%

Table 3 - Intestinal mucin production of slow-growing broilers in the starter phase (experiment 1)

Digestible threonine (%)	Mucin (g/kg excreta)
0.622	368.20
0.697	513.20
0.772	489.50
0.847	510.30
0.922	387.10
CV	4.80
Source of variation	
Linear regression	0.062
Quadratic regression	0.001

CV - coefficient of variation.

digestible threonine, which provided a maximum villus height of 1423.44 µm. The greatest crypt depth was estimated to require 0.609% digestible threonine, which resulted in a maximum crypt depth of 235.54 µm.

Threonine had a significant quadratic effect (P = 0.001) on mucin production. The increase in dietary values

Table 4 - Carcass yield and cuts of slow-growing broilers slaughtered at 50, 70, and 85 days of age in experiment 2, 3, and 4, respectively

Digestible threonine (%)	Carcass (%)	Breast (%)	TD (%)	Wing (%)	Back (%)
			Slaughter at 50 days		
0.586	63.66	29.54	34.28	13.50	22.71
0.662	64.13	31.03	33.50	13.69	21.86
0.738	64.31	30.52	34.00	13.73	21.78
0.814	64.65	29.56	34.41	13.84	22.17
0.890	64.26	30.02	34.24	13.51	22.58
CV	2.43	6.10	5.02	4.16	6.46
Source of variation			Probability		
Linear regression	0.437	0.243	0.221	0.220	0.142
Quadratic regression	0.094	0.187	0.372	0.415	0.030
			Slaughter at 70 days		
0.570	66.08	30.24	33.54	13.04	22.72
0.640	66.04	30.00	33.62	13.20	22.01
0.710	66.58	30.40	33.50	13.53	22.48
0.780	66.28	29.96	33.98	13.62	23.56
0.850	64.12	30.31	34.83	14.43	22.90
CV	5.75	4.48	5.63	7.22	7.63
Source of variation			Probability		
Linear regression	0.225	0.005	0.027	0.001	0.760
Quadratic regression	0.157	0.123	0.432	0.140	0.270
			Slaughter at 85 days		
0.520	68.69	29.23	34.66	13.13	23.00
0.595	67.75	29.28	34.49	13.39	22.88
0.670	68.71	29.70	34.03	12.80	23.48
0.745	68.43	30.05	34.09	13.36	22.53
0.820	68.89	29.34	33.76	13.49	23.40
CV	3.24	6.15	4.41	6.31	8.01
Source of variation			Probability		
Linear regression	0.547	0.460	0.022	0.356	0.818
Quadratic regression	0.453	0.338	0.130	0.226	0.802

TD - thigh and drumstick; CV - coefficient of variation.

Table 5 - Villus height, crypt depth, and villus height:crypt depth ratio of the duodenal mucosa of slow-growing broilers (experiment 4)

Digestible threonine (%)	Villus height (µm)	Crypt depth (µm)	Villus:crypt ratio (µm)
0.520	1361.47	232.14	5.86
0.595	1398.69	231.94	6.03
0.670	1408.48	232.94	6.05
0.745	1365.42	227.58	6.00
0.820	1190.27	205.24	5.80
CV	9.63	9.98	9.10
Source of variation		Probability	
Linear regression	0.064	0.081	0.072
Quadratic regression	0.001	0.002	0.002

CV - coefficient of variation.

of digestible threonine resulted in increased intestinal production of crude mucin, up to a maximum at 0.747% (Table 6) digestible threonine; at higher values of digestible threonine, mucin production decreased. The requirement estimate corresponds to a production of 415.4 g/kg excreta of intestinal crude mucin during grower phase II. There was a significant quadratic effect of dietary threonine on intestinal mucin production in the finisher phase, with an estimated 0.694% (Table 6) requirement of digestible threonine for the maximum production; this resulted in production of 410.2 g mucin/kg excreta.

Discussion

In experiment 1, the determined threonine requirements (0.716% for BWG and 0.762% for FCR) are close to the requirement of 0.78% digestible threonine that has been reported for slow-growing broilers (Cobb-Vantress, 2008), considering a similar total threonine requirement (0.90% recommended in the handbook) for digestible threonine in a corn-soybean meal-based diet and the digestibility coefficients of threonine reported by Rostagno et al. (2011).

The production of intestinal mucin ranged from 368.20 to 513.20 g/kg excreta. These values are close to those observed by Horn et al., (2009), who found values ranging from 342 to 382 g of mucin/kg excreta and below the values observed by Ospina-Rojas et al. (2013), who found from 597 to 611 g of mucin/kg excreta.

Table 6 - Intestinal mucin production of slow-growing broilers (experiment 5)

Digestible threonine (%)	Mucin (g/kg excreta)
	Grower phase II (days 50 to 69)
0.570	321.20
0.640	359.10
0.710	426.60
0.780	410.30
0.850	380.40
CV	5.20
Source of variation	
Linear regression	0.081
Quadratic regression	0.001
	Finishing phase (days 70 to 84)
0.520	355.20
0.595	389.40
0.670	418.60
0.745	396.00
0.820	385.10
CV	4.12
Source of variation	
Linear regression	0.072
Quadratic regression	0.001

CV - coefficient of variation.

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In experiments 2 and 3, with the increase in the digestible threonine values, FI decreased to a minimum and then increased. This may be related to the gradual improvement in the relationship between threonine and other essential amino acids. A physiological explanation for the increased intake observed with the increase in dietary threonine values would be a biological adjustment to meet the amino acid requirements and possible worsening of this relationship upon the increase in dietary digestible threonine (Conhalato et al., 1999). A similar effect was reported by Toledo et al. (2007), when studying the effect of lysine on the performance of broilers of conventional lines.

The estimated digestible threonine requirements for FCR (0.767% in experiment 2 and 0.733% in experiment 3) differ from the recommendation of the Nutritional Requirements for Poultry (NRC, 1994) of 0.74% total threonine during 22 to 42 days of age for broilers and from the results reported by authors who studied threonine requirements of conventional genetic lines (Duarte et al., 2012). This may be explained by the way in which the amino acid requirements are expressed in broilers (as a percentage) and by the lines compared. Conventional lines require nearly double the amount of amino acids required by slow-growing poultry; however, this difference is suppressed when observing the daily FI of broilers of conventional genetic lines (Baker and Han, 1991). This shows that the requirements of slow-growing broilers, expressed as a percentage, may be higher than those of broilers from conventional lines due to their lower daily intake.

It is noteworthy that the increase in digestible threonine led to a decrease in the breast meat yield and an increase in the thigh and drumstick yield in experiment 3. Rezaeipour and Gazani (2014), studying the effects of feed form and feed particle size with dietary L- threonine supplementation in fast-growing broilers, observed that breast and thigh yields were not affected by treatments used. This suggests that more detailed observations of the specificities of slow-growing lines with respect to the effect of this amino acid on meat cuts should be made. The interactions among threonine, lysine, and methionine can also optimize the accumulation of meat (Abudabos and Aljumaah, 2012).

In all experiments, increased digestible threonine resulted in increased TI and decreased TUE. The increased TI resulted from the increased values of feed and not from FI effects, which corroborated the findings of Corzo et al. (2007) and Mehri et al. (2010).

The findings of this study indicated that threonine has significant effects on intestinal villi, crypt depth, and intestinal mucin production. The values found for villus height are similar to those reported by Najafi et al. (2017), who studied dietary levels of threonine and found the height of villi in the duodenum ranging from 1350.33 to 1551.67 µm, and below the values found by Ospina-Rojas et al. (2013), who identified a maximum value of 1782 µm for villus height in the duodenum.

The results indicated that 0.628% digestible threonine is optimal for greater villi height. This effect may be due primarily to the direct effect of threonine on the surface area of the intestinal mucosa because threonine has higher activity in mucosal cells than does lysine or methionine (Sadrzadeh et al., 2015). Furthermore, in viscera tissues, which are drained by the portal system, threonine metabolism is higher than that of other essential amino acids (Schaart et al., 2005). Increased digestible threonine values may provide an adequate supply of this amino acid and contribute to high turnover of the intestinal mucosa (Abassi et al., 2014). Horn et al. (2009) postulated that threonine deficiency reduces intestinal protein synthesis, thereby preventing proper maintenance of the mucosa.

According to Chee et al. (2010), Rezaeipour et al. (2012), and Zaefarian et al. (2008), who observed decreased villus heights in animals with threonine deficiencies, feeds with limited threonine content results in lower villi size. Chen et al. (2016) stated that additional supplementation with L-threonine improved the intestinal morphology of broilers; this was shown by increased intestinal villus height and an increased villus height:crypt depth ratio. After the requirements for maximal villus height were met, the results showed no further increase in these parameters, which corroborated the findings of Jazideh et al. (2014), who concluded that excess threonine levels have no effect on these structures once the threonine requirements for adequate intestinal cell function are met.

The analysis of intestinal mucin production in experiments 1 and 5 showed that threonine has significant effects on mucin production and permitted the identification of requirements for maximum mucin production. Considering that increased intestinal mucin production is associated with improved nutrient use (Horn et al., 2009), identifying the conditions required for maximum production of this glycoprotein is important to ensure an intestinal environment that is protective and has adequate selectivity for optimal nutrition and animal performance.

Intestinal mucin production is very dynamic and depends on several chemical and biochemical factors. Thus, the observed gradual increase in mucin production with increasing dietary threonine values may be related to

Table 7 - Summary of threonine requirements for slow-growing broilers

Item	Estimated value (%)	R^2	Quadratic regression[1]
	Experiment 1		
Weight gain (g)	0.716	0.76	$Y = -1107.4x^2 + 1586.1x + 103.33$
Feed conversion ratio	0.762	0.60	$Y = 3.1365x^2 - 4.7828x + 3.4245$
Mucin (g/kg of excreta)	0.775	0.88	$Y = -6246.3x^2 + 9690.9x - 3234.7$
	Experiment 2		
Feed intake (g)	0.767	0.98	$Y = 11262x^2 - 17294x + 9298.1$
Feed conversion ratio	0.767	0.97	$Y = 10.223x^2 - 15.685x + 8.3158$
	Experiment 3		
Weight gain (g)	0.720	0.89	$Y = -4407.6x^2 + 6348.2x - 907.18$
Feed intake (g)	0.777	0.61	$Y = 4019.8x^2 - 6249.6x + 6584.4$
Feed conversion ratio	0.733	0.83	$Y = 13.644x^2 - 20.015x + 10.36$
	Experiment 4		
Villus height (μm)	0.628	0.96	$Y = -6064.6x^2 + 7625.7x - 973.72$
Crypt depth (μm)	0.609	0.94	$Y = -643.05x^2 + 784.14x - 3.5055$
	Experiment 5		
Mucin (g/kg of excreta) (grower phase II)	0.747	0.90	$Y = -3198.3x^2 + 4783.8x - 1373.4$
Mucin (g/kg of excreta) (finisher phase)	0.694	0.90	$Y = -1803.2x^2 + 2504.8x - 459.62$

[1] Significant regression (P<0.05).

the deficiency of this amino acid in limiting diets, which could inhibit the production of mucin glycoprotein by goblet cells. Although the mechanism by which threonine deficiency occurs is not completely understood, this amino acid is known to be essential for mucin structure (Horn et al., 2009).

Increased mucin values similar to those observed in this study after an increase in digestible threonine were also observed by Horn et al. (2009) and Ospina-Rojas et al. (2013), who concluded that threonine affects mucin production. After meeting the threonine requirements for maximum production, further increases in threonine values resulted in decreased mucin production. These results indicated that the effects of excess dietary values of the current required amounts should be further studied.

The results presented here show that the threonine requirements estimated for optimal mucin production were higher than the requirements estimated for BWG. This may be explained by the fact that threonine is of great importance in tissues of the small intestine (Hamard et al., 2007). Furthermore, this amino acid is primarily used for intestinal protein synthesis after absorption by the animal body (Kim et al., 2007); in addition, the composition of this mucus is fundamental in the digestion and absorption of nutrients (Horn et al., 2009). Therefore, the adequate supply of threonine can ensure an optimal secretion of intestinal mucus, which will contribute to a better digestibility of nutrients, directly influencing animal performance.

Thus, research studies that assess only performance parameters may underestimate the threonine requirement.

The study of the quadratic model obtained the digestible threonine requirements for all experiments with their respective determination coefficients and equations (Table 7).

Conclusions

Threonine is directly related to the maintenance of intestinal mucosa integrity and to intestinal mucin production. These parameters should be considered when estimating nutritional requirements. Values of 0.762, 0.767, and 0.733% digestible threonine may be recommended for the starter, grower I, and grower II phases, respectively, based on the best feed conversion ratio. A value of 0.694% digestible threonine may be estimated for the finisher phase based on the adequate production of intestinal mucin. The threonine values estimated for an optimal profile with lysine values lead to a digestible threonine:lysine ratio of 76.06, 53.19, and 74.26% for the starter, grower I, and grower II phases, respectively, for the best FCR and a ratio of 72.29% for the finisher growth phase, which is estimated for adequate production of intestinal mucin.

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