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Digestible Threonine Levels in the Starter Diet of Broilers Derived from Breeders of Different Ages

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ABSTRACT

The aim of this study was to evaluate the effect of digestible threonine supplementation in the starter diet on the performance, intestinal parameters, and nutrient metabolism of broilers derived from breeders of different ages. In total, 480 one-day-old Cobb chicks, derived from 38-or 49-week-oldbreeders, were housed in experimental battery cages until 21 days of age and fed four different threonine levels (800, 900, 1,000, or 1,100 mg/kg) in the starter feed. A completely randomized experimental design in a 2x4 factorial arrangement (breeder age x threonine levels) was applied, totaling eight treatments with five replicates of 12 birds each. Broilers from older breeders fed 800 mg digestible threonine/kg of diet presented higher weight gain, with a positive linear effect. There was also an interaction between breeder age and threonine levels for the weight gain of 21-d-old broilers supplemented at maximum level of 1,003 mg Thr/kg diet during the starter phase. There was no effect of breeder age or threonine levels on nutrient metabolism during the period of 17-21 days. There was no influence of breeder age or threonine levels in the starter diet on intestinal morphometric measurements, absorption area, or percentage of goblet cells.

INTRODUCTION

Breeder age can influence egg quality, composition and size, which determine the availability of nutrients to the chick during the post-hatching period (Reis *et al*., 1997; Vieira & Moran Jr., 1999). Older breeders lay larger eggs, with greater yolk and albumen contents, compared with younger ones (Peebles, 2004; Tona *et al*., 2004).

The development of the digestive tract during the first week of life of broilers is also essential for the expression of their maximum genetic potential to allow reaching optimal market weight in the shortest possible period of time (Nitsan, 1995). Therefore, any limitations during the transition period between embryonic and free life may impair the performance of broiler strains selected for rapid growth (Almeida, 2003).

Protein and amino acids perform several functions in the body, including tissue synthesis and maintenance (Alleman *et al*., 2000). Therefore, broiler feeds must be formulated to supply sufficient aminoacids and protein for synthesis; however, any crude protein excesses may reduce efficiency of amino acid utilization and increase the requirements of essential amino acids (Sklan & Plavnik, 2002).

Threonine is the third limiting amino acid for broilers after methionine and lysine in diets based on corn and soybean meal (Fernandez *et al*., 1994; Berres *et al*., 2007). It is a critical factor in least-cost formulations, and its supplementation allows reducing dietary crude protein levels,

thereby contributing to reduce uric acid and water excretion, which consequently decreases nitrogen excretion in the environment (Kidd *et al*., 2002; Kidd *et al*., 2005). Threonine is required for body protein synthesis and feather renewal, body maintenance, and collagen and elastin synthesis (Fernandez *et al.*, 1994; Sá *et al.*, 2007). It is also found in the gastrointestinal epithelium (mucosal cells, mucus, and digestive enzymes) and as a component of immunoglobulin molecules (Wu, 1998; Ojano-Dirain & Waldroup, 2002).

The threonine requirement for maintenance is high relative to the other amino acids due to its high content in endogenous intestinal secretions (Fernandez *et al.*, 1994). Faure *et al.* (2005) conducted experiments in rats and reported that dietary threonine restriction impairs the synthesis of mucins in all segments of the small intestine, with up to 40% loss in the duodenum.

Ammerman *et al.* (1995) studied threonine digestibility in poultry and concluded that it can range up to 29% among different feedstuffs, and its bioavailability is 89% in soybean meal, 84% in corn, 81% in sorghum, and 100% in L-threonine. The recommendation for digestible threonine for male broilers in the starter phase found by Rostagno *et al.* (2011) is 0,763%/3.000 kcal of metabolizable energy.

In the present study, we aimed at evaluating the effect of four different threonine levels in the starter diet (1-21 days of age) of broilers derived from breeders of two different ages on broiler growth performance, nutrient metabolism, and intestinal development parameters.

MATERIALS AND METHODS

The experiment was carried out in the facilities of the Federal University of Goiás (UFG), Goiânia, Brazil. All animal care procedures were approved before the start of the experiment by the UFG Ethics Committee in Research (protocol 15346/2012) and were in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian Society for Laboratory Animal Science (SBCAL).

In total, 480 one-day-old Cobb male chicks derived from 38- or 49-week-old breeders were fed different starter diets containing four different threonine levels(800, 900, 1,000, or 1,100 mg/kg). A completely randomized experimental design in a 2x4 factorial arrangement (breeder age x threonine levels) was applied, totaling eight treatments with five replicates of 12 birds each. Feeds were based on corn and soybean meal, and formulated to contain equal nutrient and energy levels, according to the nutritional requirement recommendations and feedstuff composition proposed by Rostagno *et al.* (2005) (Table 1). The increasing threonine levels of the experimental diets were obtained by supplementing the basal diet with L-threonine at the expense of ground corn.

Chicks were reared in cages until 21 days of age, with feed and water *ad libitum*. A program continuous light was used, providing 24 hour light. Birds and feed residues were weighed on days 4, 7, 10, 14 and 21. Weight gain (calculated as the difference between live weight at the end of each period and the initial weight at housing), feed intake (per period) and feed conversion ratio (calculated by dividing the total feed intake by weight gain, corrected for the weight of dead chicks) were calculated.

Table 1 – Ingredients and nutritional composition of the experimental starter basal diet supplemented with threonine.

Ingredients	Starter diet (kg)
Corn	61.96
Soybean meal	31.31
Soybean oil	1.83
Dicalcium phosphate	1.00
Calcitic limestone	0.84
Salt	0.44
Vitamin-trace mineral supplement*	0.50
Corn flour	1.00
L-Lysine HCL	0.67
DL-Methionine	0.30
L-Threonine	0.15
Total	100.00
Calculated nutritional composition	
Crude Protein (%)	20.50
Metabolizable energy (kcal/kg)	3.000
Calcium (%)	0.899
Available phosphorus (%)	0.449
Digestible Lysine (%)	1.434
Digestible Arginine (%)	1.210
Digestible Methionine (%)	0.576
Digestible Methionine + Cystine (%)	0.921
Digestible Tryptophan (%)	0.217
Digestible Threonine (%)	0.800
Digestible Valine (%)	0.809
Sodium (%)	0.218

*Composition/kg of feed: vitamin supplement for broilers – starter phase: vit. A – 3,125,000 IU; vit. D3 – 550,000 IU; vit. E - 3750 mg; vit. K3 - 625 mg; vit. B1 - 250 mg; vit. B2 – 1,125 mg; vit. B6 - 250 mg; vit. B12 – 3,750 mg; niacin – 9,500 mg; calcium pantothenate , 3,750 mg; folic acid - 125 mg; DL-methionine - 350,000 mg; choline chloride 50% - 150,000 mg; growth promoter - 12,500 mg; coccidiostat - 15,000 mg; Se - 50 mg; antioxidant – 2,500 mg; q.s.p. vehicle 1000 g, Mineral supplement: Fe – 100,000 mg; Cu – 16,000 mg; Zn - 100,000 mg; I – 1,500 mg.

A metabolism assay was conducted when broilers were between 17 to 21 days of age, using the method of total excreta collection. The objective was to determine the coefficient of metabolization of dry

matter (CMDM), nitrogen balance (NB), the coefficient of nitrogen metabolization (CNM), dry matter retention (DMR), and nitrogen retention (NR), according to Silva (1990). Excreta were collected twice daily, samples were placed in plastic bags duly identified per treatment and replicate, and frozen until analysis.

For the histomorphometric analysis of the digestive system, five broilers per treatment were sacrificed by cervical dislocation on days 7, 14, 21, and 28 days of age, after six hours of fasting. Three-inch segments of the duodenum, jejunum, and ileum were collected, opened by the mesenteric border, stretched by the serous tunic, washed in saline solution, and fixed in formalin at 10% for 24 hours. The tissues were washed under running distilled water, and dehydrated in increasing concentrations of alcohol (70-95%). Two changes of absolute alcohol 75% were performed within an interval of one hour each, and the intestinal fragments were embedded in paraffin at 58°C. After setting, the paraffin blocks were cut in five-micrometer slices. The sections were fixed on slides and stained by PAS technique (Luna, 1968).

Slides for histological evaluation were examined under light field optical microscope (Carl Zeiss model Juvenal) coupled to an Axio Vision 3.0 (Zeiss) image analyzing system. The captured images were further investigated by Image J software, where 40 villus height (magnification 3.2x) and crypt depth (10x magnification) measures of each intestinal segment were performed (Figures 1 and 2). Villus height was measured from the base to the tip of the villi, and crypt depth from the base of the crypt to the transition region to the villus (Fukayama *et al*., 2005). Villus to crypt ratio was calculated by dividing villus height by crypt depth.

Intestinal absorption area was calculated by measuring total villus height and width, and crypt width, with 10 readings per histological slide. All indices were analyzed using Image J software. The absorption surface area was estimated using the equation proposed by Kisielinski *et al*. (2002) as: $M= [((Wv \times Lv) + (Wv/2 + Wc/2)^2 - (Wv/2)^2) / (Wv/2 + Wc/2)^2]$, where: M = Number of times the surface of the intestinal mucosa is increased; Wv = average villus width; Lv = average villus length; and Wc = average crypt width.

Images of the intestinal mucosa were used to obtain the percentage of goblet cells in the same slides used for histomorphometry. In each slide, 10 villus sections from the duodenum, jejunum and ileum were analyzed at 10x magnification. The images were segmented by the Image J® program, in which the areas marked as goblet cells were highlighted. A quantitative analysis of goblet cells along the crypt and villus fragments was conducted. Measured areas were marked and cut in the region where there were only goblet cells, and their percentage was calculated.

Data were analyzed using the Statistical Analysis System SAS® software (Statistical Analysis System, Cary, NC, USA) using analysis of variance (GLM) and average comparison by Tukey test with a significance level of 5%.When necessary, data were submitted to polynomial regression.

RESULTS AND DISCUSSION

Feed intake, feed conversion ratio, and body weight were not influenced by the treatments ($p>0.05$). It is known that breeder age affects egg weight, quality, and composition and, consequently, chick weight (Wilson, 1991; Almeida *et al.*, 2003; Rocha *et al.*, 2008), suggesting that chicks derived from eggs laid by older breeders have more nutrients available, and therefore, those birds present better performance during the starter phase than those derived from younger breeders (Table 2).

Table 2 – Statistical probability of the effects of breeder age (BA) and dietary digestible threonine levels (Thr; mg/kg diet) on the feed intake (FI), weight gain (WG), feed conversion ratio (FCR) and body weight (BW) of 14-d-old broilers.

Treatments	FI(g/chick)	WG (g/chick)	FCR (g/g)	BW (g)
BA	0.52	<0.05	0.31	0.42
Thr	0.32	<0.05	0.22	0.38
Thr x BA	0.24	<0.05	0.18	0.26
CV (%)	4.83	3.79	4.85	3.46

Different letters in the same column differ ($p <0.05$) by Tukey's test (5%)

CV – coefficient of variation

The lowest digestible threonine level (800 mg/kg) was sufficient to meet the broiler's maintenance requirements. Stringhini *et al*. (2003) found that chick average weight at hatch influenced feed intake up to 21 days of age, but this relationship was not observed at 35 and 42 days. Higher threonine levels did not to activate the mechanisms regulating the appetite of broilers or changed the plasma amino acid profile or balance (Harper, 1970). Therefore, the brain mechanism that is sensitive to variations in amino acid blood levels and that reduces feed intake (Bertechini, 2012) was probably not stimulated in the current experiment.

The significant interaction between breeder age and digestible threonine dietary levels showed that the

addition of digestible threonine in the diet of broilers derived from young breeders had a positive linear effect on weight gain ($p<0.05$) determined on day 14, with the level of 1,000mg/kg yielding the best results (Table 3). Broilers derived from the older breeders also tended to present higher weight gain when supplemented with 1,000mg/kg. Abassi *et al.* (2014) recommended feeding broilers after 12 days of age with at least 10% higher threonine levels than those recommended by the strain's manual to increase weight gain.

Table 3 – Effect of the interaction between breeder age and digestible threonine levels (mg/kg) in the starter diet on the weight gain of 14-d-old broilers.

Breeder age (weeks)	800mg/kg	900mg/kg	1,000 mg/kg	1,100mg/kg	P (%)	CV (%)
38	367.08B	398.98	419.96	416.78	<0.05[1]	4.83
49	414.98A	413.10	415.24	411.76	0.66	4.35

Different letters in the same column differ by Tukey's test (5%) [1]Y= 239.124 + 0.1700080x / R^2 = 0.82 CV- coefficient of variation; P - statiscal probability

Feed conversion ratio and feed intake of 21-d-old broilers were not influenced by the treatments ($p>0.05$; Table 4).

The interaction between breeder age and digestible threonine levels had a quadratic effect on weight gain (Table 5), with a peak at 1,003 mg digestible threonine/kg of diet. Chickens from younger breeders presented higher weight gain when fed 1,000 mg digestible threonine/kg diet. Thus, higher digestible threonine levels than those used for the formulation of experimental diets proposed by Rostagno *et al.* (2005), of 800 mg digestible threonine/kg of diet, are recommended. The results of the present experiment do not agree with those of Reginatto *et al.* (2000), who found no effect of dietary threonine on broiler weight gain, feed intake, or feed conversion ratio, independently of dietary energy level.

Table 4 – Statistical probability of the effects of breeder age (BA) and dietary digestible threonine levels (Thr; mg/kg diet) on the feed intake (FI), weight gain (WG), feed conversion ratio (FCR) and body weight (BW) of 21-d-old broilers.

Treatments	FI (g/bird)	WG (g/bird)	FCR (g/g)	BW (g)
BA	0.34	<0.05	0.19	0.71
Thr	0.22	<0.05	0.66	0.54
Thr x BA	0.51	<0.05	0.32	0.48
CV (%)	5.27	5.98	6.13	6.57

CV – coefficient of variation

Table 5 – Effect of the interaction between breeder age and digestible threonine levels (mg/kg) in the starter diet on the weight gain of 21-d-old broilers.

Breeder Age	800 mg/kg	900mg/kg	1,000mg/kg	1,100mg/kg	P (%)	CV (%)
38	682.98	714.08	773.66 A	735.08	<0.05[1]	5.29
49	719.68	749.00	681.64 B	718.66	0.34	6.81

Different letters in the same column differ ($p<0.05$) by Tukey's test (5%).

[1]Y= -1,029.02 + 3.52568x – 0.00170080x^2; R^2 = 0.82;Maximum= 1,003 mg.

CV – coefficient of variation. p - statiscal probability

There was no effect ($p>0.05$) of breeder age or digestible threonine levels on any variable evaluated in the metabolism assay conducted during the starter phase (Table 6). It is noteworthy that all the amino acids that exceed maintenance and production requirements are catabolized, thereby reducing nitrogen retention and increasing the losses of nutrients that were not digested and utilized by the metabolism (Bertechini, 2012).

Table 6 – Metabolism of nutrients in diets supplemented with increasing digestible threonine levels (Thr; mg/kg of feed) fed to broilers derived from breeders of two different ages (BA) during the period of 17-21 days of age.

Treatments	CMDM (%)	NB (g)	CNM (%)	DMR (g/kg BW)	NR (g/kg BW)
BA	0.23	0.44	0.38	0.55	0.37
Thr	0.42	0.39	0.71	0.39	0.51
Thr x BA	0.33	0.30	0.50	0.43	0.49
CV (%)	6.62	31.84	11.09	29.48	32.46

CMDM: coefficient of metabolization of dry matter; NB: nitrogen balance; CNM: coefficient of nitrogen metabolization; DMR: dry matter retention; NR: nitrogen retention; BW: body weight. CV – coefficient of variation.

There was no effect of breeder age or digestible dietary threonine levels (p>0.05) on villus height, crypt depth, or villus:crypt ratio as measured in 14- and 21-d-old broilers (Tables 7 and 8).

Table 7 – Statistical probability of the effects of breeder age (BA) and dietary digestible threonine levels (Thr; mg/kg diet) on villus height (V; µm), crypt depth (H; µm), and villus:crypt ratio (V:C) in the duodenum, jejunum, and ileum of 14-d-old broilers.

Treatments	Duodenum			Jejunum			Ileum		
	V	C	V:C	V	C	V:C	V	C	V:C
BA	0.47	0.66	0.41	0.52	0.61	0.49	0.32	0.48	0.50
Thr	0.51	0.52	00.39	0.42	0.49	0.38	0.61	0.44	0.66
Thr x BA	0.39	0.41	00.59	0.32	0.38	0.39	0.33	0.39	0.41
CV (%)	8.01	90.56	9.23	7.41	9.16	8.92	9.57	11.03	10.84

CV – coefficient of variation

Table 8 – Statistical probability of the effects of breeder age (BA) and dietary digestible threonine levels (Thr; mg/kg diet) on villus height (V; µm), crypt depth (H; µm), and villus:crypt ratio (V:C) in the duodenum, jejunum, and ileum of 21-d-old broilers.

Treatments	Duodenum			Jejunum			Ileum		
	V	C	V:C	V	C	V:C	V	C	V:C
BA	0.32	0.29	0.41	0.33	0.40	0.38	0.41	0.28	0.47
Thr	0.33	0.38	0.55	0.45	0.35	0.51	0.39	0.32	0.50
Thr x BA	0.66	0.71	0.59	0.58	0.71	0.63	0.50	0.51	0.69
CV (%)	10.01	10.56	10.65	9.56	8.16	9.02	8.56	8.95	9.09

CV – coefficient of variation

Geyra *et al.* (2001) emphasized that crypt development influences the maintenance of crypt-cell turnover rates and intestinal maturation; therefore, the deeper the crypt, the greater the villus growth in the enterocyte, consequently maximizing intestinal absorption surface area. The similar villus heights and crypt depths measured in the intestinal segments among treatments confirm that there were no digestion and intestinal absorption improvements in the early stages of development. According to Yamauchi & Ishiki (1991), villus density is different in the various intestinal segments (duodenum, jejunum and ileum), and the number of villi is reduced when broilers are 10 days old. Those researchers concluded that this does not imply in lower absorption capacity, but in further development of the villi, and therefore, the number of villi/area is reduced with age.

The absorption area of the intestinal segments of broilers during the starter phase was not affected by dietary digestible threonine levels or breeder age (p <0.05; Table 9).

Moreover, there was no influence of breeder age or digestible threonine levels on goblet cell counts in the duodenum, jejunum, or ileum (p>0.05; Table 10). These findings are not consistent with those reported by Abassi *et al.* (2014), who showed that the jejunal histological changes observed in broilers fed increasing dietary threonine levels (100, 110, and 120%)promotes an increase in intestinal absorption surface area during the starter, grower, and finisher phases. Those authors reported that threonine influenced the number of structures, intestinal functions, and the number of goblet cells of broilers. In the digestive tract of poultry, morphological

Table 9 – Statistical probability of the effects of breeder age (BA) and dietary digestible threonine levels (Thr; mg/kg diet) on the absorption surface area (µm²) of the duodenum, jejunum, and ileum of 14- and 21-d-old broilers.

Treatments	Absorption surface area					
	Duodenum (µm)		Jejunum (µm)		Ileum (µm)	
	14 days	21 days	14 days	21 days	14 days	21 days
BA	0.46	0.44	0.56	0.52	0.49	0.61
Thr	0.55	0.33	0.41	0.60	0.59	0.69
Thr x BA	0.60	0.52	0.48	0.66	0.70	0.77
CV (%)	17.85	16.55	18.01	17.54	17.30	18.74

CV – coefficient of variation.

changes, such as increased length, height, and density of the intestinal villi, as well as higher physiological capacity, enhance nutrient digestion and absorption (Maiorka *et al*., 2002). Maiorka *et al.* (2003) stated that the absorption capacity of any intestinal segment is directly proportional to the number of villi present, villus size, and surface area available for absorption.

Table 10 – Statistical probability of the effects of breeder age (BA) and dietary threonine levels (Thr; mg/kg diet) on goblet cell counts (%) in the duodenum, jejunum, and ileum of 14- and 21-d-old broilers.

Treatments	Globet Cells					
	Duodenum (%)		Jejunum (%)		Ileum (%)	
	14 days	21 days	14 days	21 days	14 days	21 days
BA	0.64	0.55	0.51	0.60	0.71	0.80
Thr	0.62	0.66	0.42	0.52	0.51	0.70
Thr x BA	0.55	0.59	0.47	0.72	0.61	0.74
CV (%)	29.74	28.63	30.52	27.24	29.81	28.93

CV – coefficient of variation

CONCLUSION

The best performance results were obtained when broilers derived from young breeders were fed 1,000 mg digestible threonine/kg of diet and when those derived from older breeders were fed 800 mg digestible threonine/kg of diet. Digestible threonine dietary levels and breeder age did not affect nutrient metabolism or promoted any morphological changes in the intestinal mucosa during the starter phase.

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