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E-mail: editorijps@gmail.com

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Protein and Digestible Threonine Levels in Pre Starter Diets for Broiler Chicks

Alexandre Barbosa de Brito, Jose Henrique Stringhini, Roberto de Moraes Jardim Filho, Suzany Aparecida Gomes Xavier, Marcos Barcellos Cafe and Nadja Susana Mogyca Leandro
Departamento de Produção Animal, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, (UFG/EVZ), Goiânia - Goias, Brazil

Abstract: This experiment was carried out in experimental poultry facilities of University of Goias to estimate digestible threonine (Thr) requirement for broiler from 1 to 21 days of age. 384 AgRoss-508 broiler chicks were allotted in a completely randomized design in a 4 x 2 factorial arrangement, with two protein levels (205 and 221 g/kg) and four digestible threonine levels (6.1, 7.1, 8.1 and 9.1 g/kg) with four replications of 12 birds each. The performance, nutrient digestibility and morfometrical index were evaluated. The polynomial regression and linear response plateau (LRP) model was used to estimate digestible, had been the Minimum Square of Deviations (MSD) utilized for choice criterion. Used polynomial regression, the results showed that a quadratic effect ($p<0.05$) for mean body weight at seven and 21 days of age (seven days - $Y = -31.41 + 455.28x - 285.62x^2$/21 days - $Y = 510.08 + 508.91x - 324.61x^2$) which estimates a 7.9 and 7.1 g/kg of digestible Thr, but LRP model showed low MSD and smaller estimates for digestible Thr levels (7.7 and 6.7 g/kg in seven and 21 days, respectively). The results showed a tendency if lower levels of digestible Thr (below to 7.1 g/kg) reduced intestinal length. The digestible Thr levels recommended for pre-starter phase vary from 7.7 at 6.7 g/kg, estimated by LRP model and 7.9 at 7.1 g/kg estimated by polynomial regression for mean body weight at seven and 21 days of age, respectively, but the LRP values presented smaller minimum square of deviations.

Key words: Digestibility, morfometrical index, performance, protein, threonine

INTRODUCTION

Ideal protein was defined as an amino acid mixes with high digestibility, which attends animal requirements (Emmer and Baker, 1997). Lots of benefits can be obtained using this concept. Adequate ration formulas, with the most limitant amino acids, can induce to high performance results, reducing the dietetic protein and the nitrogen lost in feces, which normally determine reduced costs (Oliveira Neto and Oliveira, 2009).

Threonine (Thr) is classified as the third limiting amino acid for broilers, following sulfur amino acids and lysine, especially in low protein diets (Kidd, 2002). This amino acid has important role in protein synthesis and its catabolism generates important products like Glycine, Acetyl-CoA and Pyruvate (Kidd and Kerr, 1996). According to Samadi and Liebert (2007), threonine requirements must consider the protein level of the ration and in high-protein diets can be considered one of the most important essential amino acids in poultry body protein deposition, which must consider the age and growing taxes.

Soares *et al.* (2000) estimated digestible Thr levels in 7.3 g/kg for broilers from 1 to 21 days of age. Rosa *et al.* (2001) obtained 7.1 and 7.2 g/kg for males and females, in initial period, respectively. For Albino *et al.* (2002), the best level required by chicks from 1 to 20 days of age was 7.9 g/kg with relationship Lys:Thr in ratio of 69%. It is possible to observe that Thr levels are varied, which difficult interpretation.

These differences are related to age, sex, protein level, interaction with other amino acids and the methodology adopted to estimate animal requirements (Webel *et al.*, 1996; Barkley and Wallis, 2001). According to Sakomura and Rostagno (2007), polynomial regression and Linear Response Plateau Model (LRP) are appropriate to estimate amino acid requirements.

This experiment aimed to evaluate the effect of digestible Thr and protein supplementation in pre-starter period (one to seven days of age) on performance, digestibility, nutrient retention and morphometrical indexes for broilers at 21 days old.

MATERIALS AND METHODS

This experiment was carried out in experimental poultry facilities of the Veterinary College of the University of Goias (Goiania, Brazil) with 384 AgRoss-508 one-day old chicks.

The birds, males and females, were allotted in brooded batteries of 0.6 m^2. The mean temperatures measured inside the house were 30.5±3.2°C during the first week and 27.5±7.3°C in the starter period (eight to 21 days).

Corresponding Author: Alexandre Barbosa de Brito, Departamento de Producao Animal, Escola de Veterinária e Zootecnia, Universidade Federal de Goias, CP 131, CEP 74.001-970, Goiania, Goias, Brazil

Table 1: Dietary ingredients (g/kg) and chemical composition of experimental diets used for broilers fed different protein and digestible Threonine (Thr) levels in pre-starter ration (one to seven days) and starter (eight to 21 days)

	Pre-starter ration								
	205 g/kg of crude protein				221 g/kg of crude protein				
Ingredients (g/kg)	6.1 g/kg of Thr	7.1 g/kg of Thr	8.1 g/kg of Thr	9.1 g/kg of Thr	6.1 g/kg of Thr	7.1 g/kg of Thr	8.1 g/kg of Thr	9.1 g/kg of Thr	Starter ration
Corn	645.00	645.00	645.00	645.00	525.00	525.00	525.00	525.00	586.40
Soybean meal	230.00	230.00	230.00	230.00	59.80	59.80	59.80	59.80	371.00
Corn gluten meal	40.40	40.40	40.40	40.40	165.70	165.70	165.70	165.70	0.00
Wheat meal	31.30	31.30	31.30	31.30	190.90	190.90	190.90	190.90	0.00
Dicalcium phosphate	13.70	13.70	13.70	13.70	12.40	12.40	12.40	12.40	19.70
Limestone	14.30	14.30	14.30	14.30	16.00	16.00	16.00	16.00	9.50
DL-Methionine 99	2.80	2.80	2.80	2.80	1.40	1.40	1.40	1.40	0.90
L-Lysine HCL	5.70	5.70	5.70	5.70	9.20	9.20	9.20	9.20	0.00
L-Threonine	0.00	1.00	2.00	3.00	0.00	1.00	2.00	3.00	0.00
L-Triptophan	0.10	0.10	0.10	0.10	0.60	0.60	0.60	0.60	0.00
L-Arginine	2.00	2.00	2.00	2.00	4.00	4.00	4.00	4.00	0.00
Common salt	4.70	4.70	4.70	4.70	5.00	5.00	5.00	5.00	2.70
Min/Vit Supl.[1]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.80
Inert[2]	5.00	4.00	3.00	2.00	5.00	4.00	3.00	2.00	0.00
Nutrients	Calculated composition[3]								
Crude protein, g/kg	205.0	205.0	205.0	205.0	221.0	221.0	221.0	221.0	211.0
AMEn[4], MJ/kg	12.7	12.7	12.7	12.7	12.7	12.7	12.7	12.7	13.0
Dig[5]. Lysine, g/kg	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.1
Dig[5]. Met+Cys, g/kg	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	7.9
Dig[5]. Threonine, g/kg	6.1	7.1	8.1	9.1	6.1	7.1	8.1	9.1	6.5
Dig[5]. Triptophan, g/kg	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.8
Dig[5]. Arginine, g/kg	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	11.9
Calcium, g/kg	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.6
Available phosphorus, g/kg	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	4.8
Sodium, g/kg	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	1.5

[1]Mineral and Vitamin supplement added per kg of diet: 2.5 mg retionol, 75 mg cholecalciferol, 5 mg a-tocopherol acetate, 2 mg menadione sodium bisulfite, 1 mg thiamine, 4 mg riboflavin, 2 mg pyridoxine, 10 mg cyanocobalamin, 1 mg folic acid, 10 mg niacin, 10 mg Ca pantothenate, 30 mg biotin, 150 mg choline, 50 mg Mn, 50 mg Zn, 50 mg Fe, 600 mg Mo, 500 mg Co, 600 mg I, 4 mg Cu, 70 mg Se, 80 mg BHT.
[2]Rice hulls. [3]Values calculated by feed composition proposed by Rostagno (2000). [4]Aparent Metabolizable Energy. [5]Digestible

The experimental diets included two protein levels (205 and 221 g/kg) and four digestible threonine levels (6.1, 7.1, 8.1 and 9.1 g/kg) and isoenergetic and isonutritious for other nutrients; the nutritional composition and nutritional requirements were based in Rostagno (2000) recommendations (Table 1). The Lys:Thr relationship were respectively: 100:52, 100:60, 100:68 and 100:77. During starter period (8 to 21 days), all birds received the same starter ration (Table 1). All diets were corn and soybean based.

Birds were allotted in a completely randomized design in a factorial arrangement 2 x 4 (protein x digestible threonine levels) with four replicates of 12 birds each.

The chicks were weighed in 1st, 4th, 7th and 21st days of age to calculate mean weight gain, feed intake, feed-to-gain ratio and mortality. From the 4th to the 7th days of age, the total excreta produced were collected in intervals of 12 h and refrigerated until the chemical analysis. Was utilized the total excreta collection method as described by Sakomura and Rostagno (2007) and in the Animal Nutrition Laboratory of the Veterinary College of the University of Goias, all samples were prepared to for the evaluation of dry matter and nitrogen values, had been generated the digestibility coefficient and nutrient retention values (expressed in grams and milligrams per weight gain) were calculated according to Noy and Sklan (2002).

At 7th and 21st days of age, one bird per replicate was sacrificed in the Ornitopathology Laboratory of the Veterinary College of the University of Goias, following the methods proposed by Brazilian Council for Animal Welfare (Cobea, 1979) to evaluate intestines weight and length, oesophagus + crop, proventriculus + gizzard, liver + gall bladder and pancreas relative weight. All morphometrical data were expressed in percentage of body weight.

Digestible Thr requirement was estimated by polynomial regression and LRP model and Minimum Square of Deviations (MSD) used as criterion to indicate the best levels, but for protein levels the Tukey test (5%) was used. The statistical analyses were accomplished by SAEG program (UFV/SAEG, 2000).

RESULTS

The mean body weight at first day was 42.5±0.7 g. No statistical interaction was observed for Thr levels and protein (Table 2). A quadratic effect ($p<0.05$) was verified for mean body weight at seven days

Table 2: Mean Body Weight (BW), Feed Intake (FI), feed-to-gain ratio (FCR) and Mortality Rate (Mort) at seven days for broilers fed increasing levels of digestible threonine and two protein levels in pre-starter ration

Treatments		BW (g)	FI (g)	FCR (g)	Mort (%)
Prot (g/kg)	205	145.38	113.85	1.14	2.84
	221	146.90	117.72	1.13	0.57
Thr (g/kg)	6.1	140.53	112.18	1.17	2.27
	7.1	147.83	116.29	1.13	2.27
	8.1	150.17	119.64	1.12	1.14
	9.1	146.04	115.02	1.13	1.14
CV[1] (%)		3.81	5.99	4.30	17.94
Probability					
Thr		1.41	21.72	27.30	>50.00
Prot		>50.00	12.72	>50.00	15.11
Thr x Prot		>50.00	>50.00	>50.00	34.55

[1]Coefficient of variation

Table 3: Mean Body Weight (BW), Feed Intake (FI), feed-to-gain ratio (FCR) and Mortality Rate (Mort) at 21 days for broilers fed increasing levels of digestible threonine and two protein levels in pre-starter ration

Treatments		BW (g)	FI (g)	FCR (g)	Mort (%)
Prot (g/kg)	205	704.25	880.43	1.42	5.00
	221	706.47	894.08	1.41	1.25
Thr (g/kg)	6.1	699.32	883.47	1.39	2.50
	7.1	710.55	888.00	1.42	5.00
	8.1	706.66	891.43	1.43	2.50
	9.1	704.91	886.12	1.41	2.50
CV[1] (%)		5.64	4.38	2.98	14.40
Probability					
Thr		4.68	>50.00	36.66	>50.00
Prot		>50.00	>50.00	>50.00	7.76
Thr x Prot		>50.00	>50.00	25.14	13.52

[1]Coefficient of variation

Table 4: Digestibility and retention indexes for Dry Matter (DM). Crude Protein (CP) calculated for broilers fed increasing levels of digestible threonine and two protein levels in pre-starter ration

		Digestibility		Retention	
Treatments		DM (g/kg)	CP (g/kg)	DM (mg/g)	CP (mg/g)
Prot (g/kg)	205	774.80	694.90	892.93	27.20
	221	772.40	694.80	876.23	27.10
Thr (g/kg)	6.1	768.50	682.90	888.76	26.75
	7.1	773.80	711.50	874.28	27.94
	8.1	771.80	688.10	862.85	26.48
	9.1	780.40	696.80	912.43	27.43
CV[1] (%)		2.86	5.28	6.11	7.62
Probability					
Thr		>50.00	>50.00	>50.00	>50.00
Prot		>50.00	>50.00	31.24	>50.00
Thr x Prot		18.29	>50.00	>50.00	>50.00

[1]Coefficient of variation

($Y = -31.41 + 455.28x - 285.62x^2$, $R^2 = 0.88$) was estimated a 7.9 g/kg of digestible Thr in the maximum point of the curve and a 7.08 of MSD. No effect was detected for other performance variables ($p>0.05$).

For digestible threonine levels from one to 21 days of age (Table 3), a quadratic effect ($p<0.05$) was observed for mean body weight at 21 days ($Y = 510.08 + 508.91x - 324.61x^2$, $R^2 = 0.89$) was estimated a 7.1 g/kg of digestible Thr in the maximum point of the curve and a 6.12 of MSD. No effect was detected for other performance variables ($p>0.05$). When mean body weight at seven and 21 days of age were analyzed by LRP curves (Illustration 1), the values estimated for digestible threonine were lower than polynomial regression (7.7 and 6.7 g/kg, respectively) and lower values for MSD (4.08 and 1.53, respectively).

For nutrient digestibility and retention (Table 4), no effect of digestible Thr and protein was observed ($p>0.05$). For organ indexes at seven days of age (Table 5), no difference was observed ($p>0.05$), but for organ indexes at 21 days of age (Table 6) there was a statistical tendency for interaction among digestible Thr levels and Protein for intestinal length ($p = 0.0513$).

DISCUSSION

According to Kidd *et al.* (2001), broilers had good response to 6.9 and 7.0 g/kg of digestible threonine in starter phase when weight gain and feed-to-gain ratio was evaluated, the authors observed a dose-response effect, resulting in a quadratic distribution of data always increase the levels above the mentioned limits. But, broilers can have different responses to breast meat deposition for higher levels of Thr. This may be a tendency to study separately performance and breast meat yield considering digestible Lys:Thr ratio (Williams, 1995; Kidd *et al.*, 1997; Atencio *et al.*, 2004; Berres *et al.*, 2007).

Dozier *et al.* (2004) evaluated nitrogen retention in broilers, males and females, fed 180 g/kg of crude protein diets with low (5.2 g/kg) and adequate digestible Thr levels (7.4 g/kg) didn't observe differences ($p>0.05$) for the nutritional levels tested. Males that consumed 7.4 g/kg Thr had higher nitrogen retention than those fed 5.2 g/kg Thr diets (38.8 and 21.5 mg/g, respectively). No difference was obtained for females.

Shan *et al.* (2003) supplemented increasing levels of threonine for starter rations (1 to 21 days) on two different allotment temperatures (25 and 35°C) and estimated the digestible Thr requirements in 7.3 and 7.2 g/kg for weight gain and feed intake, respectively, using the LRP method. The temperature influenced feed-to-gain ratio and the results indicated better results for 7.4 at 25°C and 7.2 g/kg of digestible Thr for 35°C.

Ciftci and Ceylan (2004) tested the supplementation of Thr (6.0 to 11.0 g/kg of digestible Thr) and 170 and 190 g/kg protein in starter (1 to 21 days) and growing diets (22 to 40 days). The authors observed statistical interaction between protein and digestible threonine for feed intake and mean body weight and the best digestible Thr levels estimated by LRP was 7.1 and 6.9 g/kg for starter and growing phases, respectively.

Recently, Zaghari *et al.* (2011) verified that levels from 0.65-0.90% are enough to guarantee the best performance and gut functionality in broiler diets

Table 5: Intestinal Length (INTL) and relative weight of Crop + Oesophagus (CROPE), Proventriculus + Gizzard (PROVG), Liver (LIV), Pancreas (PANC) and Total Gastrointestinal Tract (TGIT) at seven days for broilers fed increasing levels of digestible threonine and two protein levels in pre-starter ration

Treatments		INTL (cm)	CROPE (%)	PROVG (%)	LIV (%)	PANC (%)	TGIT (%)
Prot (g/kg)	205	80.06	1.46	7.27	4.57	0.46	7.07
	221	82.88	1.41	7.15	4.32	0.48	7.03
Thr (g/kg)	6.1	78.56	1.46	7.24	4.71	0.48	6.80
	7.1	82.69	1.33	6.60	4.14	0.45	6.85
	8.1	78.25	1.43	7.47	4.32	0.46	7.27
	9.1	86.38	1.53	7.52	4.60	0.49	7.28
CV[1] (%)		7.66	10.62	13.38	11.55	7.81	11.55
Probability							
Thr		5.89	8.97	22.77	13.15	>50.00	>50.00
Prot		21.51	>50.00	>50.00	17.10	>50.00	>50.00
Thr x Prot		>50.00	7.05	>50.00	17.38	>50.00	>50.00

[1]Coefficient of variation

Table 6: Intestinal Length (INTL) and relative weight of Crop + Oesophagus (CROPE), Proventriculus + Gizzard (PROVG), Liver (LIV), Pancreas (PANC) and Total Gastrointestinal Tract (TGIT) at 21 days for broilers fed increasing levels of digestible threonine and two protein levels in pre-starter ration

Treatments		INTL (cm)	CROPE (%)	PROVG (%)	LIV (%)	PANC (%)	TGIT (%)
Prot (g/kg)	205	105.63	0.85	3.42	2.77	0.32	3.94
	221	106.16	0.84	3.37	2.80	0.29	3.91
Thr (g/kg)	6.1	110.38	0.81	3.42	2.93	0.33	4.15
	7.1	102.69	0.75	3.46	2.80	0.29	3.81
	8.1	105.75	0.97	3.33	2.75	0.31	3.92
	9.1	104.75	0.85	3.39	2.67	0.30	3.81
CV[1] (%)		8.79	11.98	11.67	9.29	7.20	6.80
Probability							
Thr		>50.00	15.18	>50.00	24.93	>50.00	5.87
Prot		>50.00	>50.00	>50.00	>50.00	16.24	>50.00
Thr x Prot		5.13	26.03	>50.00	>50.00	>50.00	23.53

[1]Coefficient of variation

containing 17.5% crude protein from 0 to 21 days of age, which agrees with the Threonine levels determined in this experiment.

Conclusion: The digestible Thr levels recommended for pre-starter phase vary from 7.7 at 6.7 g/kg, estimated by LRP model and 7.9 at 7.1 g/kg estimated by polynomial regression for mean body weight at seven and 21 days of age, respectively, but the LRP values presented smaller minimum square of deviations. For other analyzed variables no effect of digestible Thr and protein was observed (p>0.05).

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