



Threonine-lysine ratio on the requirements of digestible lysine in diets for broilers

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ABSTRACT. Two experiments were performed to determine the influence of threonine-lysine (Thr:Lys) ratio on requirements of digestible lysine (Lys) in low crude protein diets for broilers in the growth phase. In the first experiment, a total of 480 Cobb 500 male broilers were distributed in a completely randomized experimental design with four dietary Thr:Lys ratio (57.0; 60.5; 64.0 and 67.5%) and with five replicates of 24 birds each. No significant differences were reported in weight gain, feed intake, poultry manure humidity, carcass and meat yields. However, the feed conversion was increased linearly as dietary Thr:Lys ratio was increased. In the second experiment, a total of 400 Cobb 500 male broilers were distributed in a completely randomized experimental design with four digestible Lys levels (1.005, 1.045, 1.085 and 1.125%), and with four replicates of 25 birds each. No significant differences were reported in performance, poultry litter humidity and carcass and meat yields. Lys levels of 1.005% and Thr:Lys at 57% were sufficient to maximize the performance and carcass yield of broilers during the growth phase when fed on low crude protein diets.

Keywords: amino-acid, lysine, ideal protein, threonine.

Relação treonina: lisina sobre a exigência de lisina digestível em dietas para frangos de corte

RESUMO. Dois experimentos foram realizados com o objetivo de determinar a influência da relação treonina:lisina (Thr:Lys) sobre a exigência de lisina (Lys) digestível em dietas com baixa PB para frangos de corte na fase de crescimento. No primeiro experimento, foram utilizados 480 frangos de corte, machos da linhagem Cobb 500, distribuídos em um delineamento experimental inteiramente casualizado, com quatro relações de Thr:Lys (57,0; 60,5; 64,0 e 67,5%) e cinco repetições com 24 aves cada. Não foram observadas diferenças significativas no ganho de peso, consumo de ração, umidade de cama e rendimento de carcaça e de cortes. Entretanto, a conversão alimentar piorou conforme a relação Thr:Lys aumentou na dieta. No segundo experimento, foram utilizados 400 frangos de corte distribuídos em um delineamento experimental inteiramente casualizado, com quatro níveis de Lys digestível (1,005; 1,045; 1,085 e 1,125%) e quatro repetições com 25 aves cada. Não foram observadas diferenças significativas no desempenho, umidade de cama e rendimento de carcaça e de cortes. O nível de Lys de 1,005% e a relação de Thr:Lys de 57% foi suficiente para maximizar o desempenho e o rendimento de carcaça de frangos de corte na fase de crescimento alimentados com dietas baixas em PB.

Palavras-chave: aminoácido, lisina, proteína ideal, treonina.

Introduction

The commercial availability of threonine (Thr) in poultry breeding has provided diet formulation of broilers with lower crude protein (CP) levels than those normally employed by the industry. The maximum level in which diet protein may be reduced without compromising the performance of broilers should be investigated.

Thr is not exclusively employed for protein deposition since it is also involved in other basic metabolic processes. The amino-acid participates in the synthesis of feather protein (STILBORN et al., 1997)

and it is the precursor of glycine and serine through the activities of the Thr enzyme aldolase and desidrogenase (CORZO et al. 2009a). Thr is highly efficient in immune response since it is the main amino-acid in the gamma serum immunoglobulin (BHARGAVA et al., 1971), it participates in the formation of collagen and elastin (SÁ et al., 2007) and indirectly favors decrease of thermal stress when CP is reduced in the diet (LEMME, 2001).

Although Rostagno et al. (2011) recommend Thr:Lys 65% for broilers from 22 - 42 days old, Atencio et al. (2004) reported a lower Thr:Lys ratio,

with 60%, when broilers were 24 - 38 and 44 - 56 days old. On the other hand, Berres et al. (2007) determined Thr:Lys 63.5% at age 15 - 37 days.

Although Thr is the third limiting amino-acid in corn and soybean bran-based diets for broilers, the literature is contradictory with regard to this requirement, especially during the 22 - 42 days after birth when Thr is relevant for maintenance. In fact, the amino-acid makes up the mucin glycoprotein (FAURE et al., 2005) and its high concentrations of endogenous secretion concentrations, whereas non re-used Thr is contained in the secretions by the birds (BURRIN et al., 2001; LI et al., 1994).

Robbins (1987) and more recently Zaghari et al. (2011) reported that Thr requirements for weight gain are higher in broilers fed on CP-rich diets than in those fed on low CP diets. However, Ciftci and Ceylan (2004) stated that Thr requirements are higher in low CP diets.

Moreover, lysine (Lys) requirements are also affected by CP in the diet, similar to Thr (DOZIER III et al., 2008). Lys is the first limiting amino-acid in diets for broilers with protein decrease, when soybean bran is not the main protein source in the diet (AHMAD et al., 2007). It is highly important to better understand digestible Thr and Lys requirements and their respective relationship in low protein diets formulated to comply with the minimum requirements of essential amino-acids to decrease diet costs and nitrogen excretion.

Current research determines the influence of ideal ratio Thr:Lys on the requirements of digestible Lys in low crude protein diets for 22 - 42-day-old broilers.

Material and methods

The two experiments were performed in the poultry sector of the Iguatemi Experimental Farm of the State University of Maringá, Maringá, PR, Brazil. The first experiment comprised 480 male broilers, commercial Cobb 500 strain, distributed in a completely randomized experimental design with four digestible Thr:Lys (57.0, 60.5, 64.0 and 67.5%) and five replications of 24 broilers per experimental unit. The second experiment comprised 400 commercial Cobb 500 broilers, distributed in totally randomized experimental designs, with four digestible lysine levels (1.005, 1.045, 1.085 and 1.125%) and four replications with 25 broilers per experimental unit.

One-day-old birds were placed in a 15 m by 8 m conventional shed with 2.5 boxes, with continuous illumination during the first week and with 23 hours

light period at the end of the experimental period. First-use rice husk was used for poultry litter whilst water and experimental diets were given *ad libitum*.

Broilers received a conventional diet from the first day of birth till they were 21 days old and experimental diets from 22 to 42 days old. Diet consisted of corn and soy meal formulated with food chemical composition rates and nutritional requirements for male broilers in each phase, following Rostagno et al. (2005).

Iso-nutrition and iso-energetic diets were provided during the growth phase (22-42 days), with variations in Thr and Lys contents in the 1st and 2nd experiments, respectively, following ideal protein standard proposed by Rostagno et al. (2005) for digestible amino-acids (Table 1). Diets in the second experiment were formulated with digestible Thr:Lys ratio found to be ideal in the first experiment.

Table 1. Percentage composition of basal diet in the first (different Thr: digestible Lys ratio) and second (different levels of digestible Lys) experiments.

Ingredients, %	Experiment	
	1	2
Corn	72.00	71.70
Soybean meal 45%	22.60	22.70
Soybean oil	1.470	1.700
Dicalcium phosphate	1.600	1.620
Limestone	1.060	1.080
Common salt	0.390	0.391
DL-Methionine (99%)	0.258	0.228
L-Lysine HCL (98%)	0.420	0.365
L-Threonine (98%)	0.044	0.021
L-Tryptophan (98%)	0.010	-
Mineral and vitamin supplement ^{1,2}	0.150	0.200
Nutritional rates		
Crude protein (%)	17.00	17.00
Metabolizable energy (kcal kg ⁻¹)	3.100	3.100
Calcium (%)	0.875	0.874
Sodium (%)	0.190	0.192
Available phosphorus (%)	0.402	0.406
Digestible lysine (%)	1.050	1.005
Methionine + digestible cystine (%)	0.762	0.724
Digestible threonine (%)	0.600	0.573

¹Growth Vitamin mixture (contents per kg of premix): vit. A - 6,000,000.00 UI; vit. D3 - 2,000,000.00 UI; vit. E - 10,000.00 mg; vit. K3 - 1,000.00 mg; vit. B1 - 1,400.00 mg; vit. B2 - 4,000.00 mg; vit. B12 - 10,000.00 mcg; niacin - 30,000.00 mg; pantothenic acid - 11,000.00 mg; folic acid - 600.00 mg; antioxidant - 100,000.00. ²Mineral mixture (contents per kg of premix): Fe - 10,000.00 mg; Cu - 16,000.00 mg; I - 2,400.00 mg; Zn - 100,000.00 mg; Mn - 140,000.00 mg; Se - 400.00 mg.

Weight gain, diet intake and feed conversion during the whole experimental period were evaluated to determine the broilers' performance. Broilers and experimental diets were weighed on 21, 28, 35 and 42 days of age. Broilers' mortality was reported daily and possible causes were determined after necropsy.

Two broilers per experimental unit (10 birds per treatment) were used to determine carcass yield at 42 days old. The birds were selected according to the replication's average weight, with a six-hour fast, stunned with electric charge and killed by jugular bleeding.

Eviscerated carcass weight, without feet, head and belly fat, was used to calculate carcass yield, and compared to live weight of broilers weighed before killing. Yield of whole breast with skin and bones, and legs were taken into account for the yield of noble parts, calculated and compared to weight of eviscerated carcass.

Samples with approximately 500 g of poultry litter samples were collected at three sites per box at the end of the experimental period, keeping a minimum distance of 10 cm from water and meal troughs. Samples were homogenized and stored for dry matter analysis.

Statistical analyses of the characteristics under analysis were undertaken by the system program for statistic and genetic analysis (SAEG, 2001). Digestible Thr: Lys ratio and digestible Lys were established with polynomial regression models.

Results and discussion

Thr-Lys ratio did not affect weight gain, diet intake, poultry litter humidity and carcass and meat yield on broilers fed on low CP diets (Tables 2 and 3). According to the above, 0.60% level of digestible Thr, equivalent to 57% Thr-digestible Lys ratio, was sufficient to maximize the performance of male broilers fed on diets with 17.0% crude protein. Thr-digestible Lys ratio, determined as ideal in current analysis, was similar to that estimated by Soares et al. (1999) with regard to 57% for Hubbard male broilers within the 22 - 42 day phase, with 0.57% Thr requirements.

Table 2. Performance and poultry litter humidity of broilers fed on low CP diets and different Thr:digestible Lys ratio for 22 - 42 days of age.

Ratio Thr:Lys (%)	Weight gain (g)	Feed intake (g ave ⁻¹)	Feed conversion (g g ⁻¹) ¹	Humidity (%)
57.0	1826.22	3658.28	2.004	22.69
60.5	1814.72	3643.38	2.007	26.34
64.0	1723.00	3538.24	2.054	28.23
67.5	1780.49	3671.24	2.062	25.81
CV (%)	4.12	3.53	1.34	12.49

¹Linear effect ($p < 0.05$). CA = $1.63635 + 0.00635x$.

Table 3. Carcass and meat yield of broilers fed on low CP diets and different Thr:digestible Lys at 42 days of age*.

Thr:Lys ratio (%)	Carcass (%)	Leg (%)	Breast (%)
57.0	70.85	29.12	29.79
60.5	70.14	30.25	28.19
64.0	71.24	29.08	31.45
67.5	71.30	29.33	29.97
CV (%)	2.81	5.06	9.24

*not significant ($p > 0.05$).

Similarly, Atencio et al. (2004) did not report any effects of Thr levels on broilers' performance in diets with 19.9 and 17.8% CP respectively in 24 - 38

and 44 - 56-day old broilers. It seemed that diets with 0.637 and 0.565% Thr digestible levels, equivalent to a 60% ratio in the two phases, were sufficient for broilers' requirements.

Berres et al. (2007) likewise did not determine the effect of Thr level on the performance and yield of carcass and meat of broilers fed on diets with 18.5% CP between 15 and 37 days old, determining a 63.5% ratio of Thr:Lys. Thr:Lys ratios, considered ideal in current study and in research above, are lower than those established by Rostagno et al. (2011) featuring 65% in broilers during the growth and finishing phase. The latter is even lower to ratio 70% (0.74% of Thr) by Kidd et al. (1996) with a diet of 19% CP for broilers within the 21 - 42 days of age.

According to Zaghari et al. (2011) and Robbins (1987), Thr requirements for maximum weight gain is less in broilers fed on low CP diets than those fed on CP-rich diets. Rangel-Lugo et al. (1994) calculated Thr requirements for 0.67 and 0.77% weight gain in broiler chickens fed on a diet with 20 and 25% CP respectively. In fact, CP rate in the diet is one of the main factors that affect Thr requirements (AHMADI; GOLIAN, 2010).

Consequently, Thr:Lys ratios may be less in diets with low protein contents. CP-rich diets produce greater nitrogen excretion in the form of uric acid which increases glycine requirements since the amino-acid composes the uric acid molecule (SONNE et al., 1946). Since Thr is the precursor of glycine (CORZO et al., 2009a; OSPINA-ROJAS et al., 2013), CP-rich diets also increase Thr requirements.

Soares et al. (1999) and Duarte et al. (2012) determined the ratio Thr:Lys at 57 and 71%, respectively, even though they used the same commercial strains, equal experimental age and diets were based on corn and soy meal. According to Kidd et al. (2003), Thr requirements may vary according to the importance of different metabolic functions in which the amino-acid participates. Thr requirements may depend on breeding conditions provided for broilers.

Further, Corzo et al. (2007) reported digestible Thr requirements for weight gain which were higher in broilers bred in reused poultry litter than in non-reused one. Likewise, Kidd et al. (2003) estimated higher digestible Thr requirements in broilers bred in a contaminated environment than those maintained in a clean one. Both studies showed that increase in Thr

requirements was due to greater needs for the maintenance of the gastro-intestinal system in these conditions.

According to Zaghari et al. (2011), Thr highly affects the development and adequate functioning of broilers' intestine since it is the main amino-acid in mucins (FAURE et al., 2005). In fact, they have an important role in the protection of the intestine by protecting it from chyme acidity, the activities of digestive enzymes and pathogenic agents (HORN et al., 2009). Low Thr:Lys ratio, determined as ideal in current study, may be due to low immunity challenge in experimental birds by decreasing Thr use in the renewal of intestine mucus and increasing its disposition for the addition of tissue protein.

Soybean meal in the formulation of diets for broilers greatly satisfies the requirements of digestible Thr due to its high contents of the amino-acid (ROSTAGNO et al., 2011). Therefore, the supplementation of industrial Thr reduces the inclusion of soy meal and, consequently, the costs of diets and minimizes CP levels in the diet (KIDD et al., 2002). However, gradual decrease of CP in the diet may cause deficiency of amino-acids, such as valine, isoleucine, arginine and tryptophan, known in the literature as "less limiting", which are generally supplemented by diets with normal CP levels (PEGANOVA; EDER, 2002).

Feed conversion in current analysis worsened linearly by gradual increase of Thr-Lys ratios. The above results indicate an amino-acid imbalance in experimental diets. Diets with reduced protein and deficient in one or more amino-acids cause the deamination of excess dietetic amino-acids (Thr), coupled to the resultant nitrogen excreted as uric acid. Since the process demands energy expenditure, it is a negative factor in the broilers' performance.

According to Wang et al. (2007), adequate provision of Thr in the diet is highly relevant in the maintenance of the gastro-intestine tract and to provide a synthesis of tissue protein. However, excess of diet Thr impairs protein synthesis in the extra-hepatic tissues and their respective growth and development. The animals' low productive performance results.

No effect ($p > 0.05$) of Lys level was reported on weight gain, poultry litter humidity and broilers' carcass and meat in birds fed on low CP diets (Tables 4 and 5). Consequently, 1.005% level of digestible Lys was sufficient to maximize the performance of male broilers fed on diets with

17.0% CP and maintain the ratio Thr:Lys at 57%. However, Rostagno et al. (2011) recommended higher digestible Lys levels, or rather, 1.044% for broilers during the growth phase fed on a diet with 18.75% CP.

Lys requirements increased linearly with the rise of CP levels in the diet (ABEBE; MORRIS, 1990; MORRIS et al., 1999). The above may explain lower digestible Lys level determined as ideal in current experiment since the broilers received a low protein diet.

Table 4. Performance and poultry litter humidity of broilers fed on low CP diets and different levels of digestible Lys at 22 - 42 days old*.

Digestible Lys (%)	Weight gain (g)	Feed intake (g bird ⁻¹)	Feed conversion (g g ⁻¹)	Humidity (%)
1.005	1749.70	3592.20	2.053	35.35
1.045	1723.00	3572.20	2.075	32.93
1.085	1746.20	3598.52	2.061	35.74
1.125	1690.71	3471.49	2.054	32.05
CV (%)	2.50	2.03	1.83	10.62

*not significant ($p > 0.05$).

Table 5. Carcass and meat yield of broilers fed on low CP diets and different digestible Lys levels at 22 - 42 days old*.

Dig Lys (%)	Carcass (%)	Leg (%)	Breast (%)
1.005	69.83	30.57	33.02
1.045	67.85	31.31	31.60
1.085	68.31	30.73	32.27
1.125	69.48	30.72	32.47
CV (%)	1.95	5.30	4.73

*not significant ($p > 0.05$).

Several studies have shown that Lys affected positively broilers' carcass yield (ABUDADOS; ALJUMAAH, 2010; CORZO et al., 2006; DOZIER III et al., 2008;), mainly breast yield, due to the fact that the concentration of the amino-acid lies in the *Pectoralis major* and *Pectoralis minor* muscles (MUNKS et al., 1945). However, level of digestible Lys on breast yield was not registered. Corzo et al. (2009b) reported that Lys and Thr requirements and their respective ratio must be determined simultaneously in the same experimental conditions to minimize the experimental variability of the animals (matrixes, incubation conditions), ingredients (nutrient concentrations, digestibility, bioavailability) and environmental conditions (relative humidity, temperature, sanitary challenges).

Although Thr is a highly analyzed amino-acid, further research work on its requirements and its ratio with Lys are needed since studies still demonstrate contradictory results even when the same ingredients in experimental diets and the same commercial strains are employed. The situation mentioned above is partially due to numberless metabolic processes in which Thr

participates, to CP contents in the diet and to constant genetic changes in broilers' commercial strains which may affect the requirements of all amino-acids.

Conclusion

Within the conditions in which current experiment was performed, level of digestible lysine at 1.005%, with ratio threonine:lysine at 57%, was sufficient to maximize the performance and yield of carcass and commercial meat in low CP diets for broilers within the 22 - 42 days old phase.

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