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NONRUMINANT NUTRITION

Threonine-to-lysine ratio in laying hens: physiological parameters and organ weight

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ABSTRACT. The objective of this study was to evaluate the effect of threonine:digestible lysine ratio in the diet on the physiological variables and weight of organs of light laying hens. Two hundred and ten 47 week-old Dekalb White laying hens were distributed in a completely randomized design, with five levels of threonine (0.507; 0.552; 0.597; 0.642 and 0.677%) and seven replicates of six birds each. The experimental period was 10 weeks, totaling 62 days and more eight days for the animals to adapt. The physiological parameters of cloacal temperature (CT), respiratory rate (RR) and average surface temperature (AST) were recorded weekly (7:00 am, 10:00 am, 1:00 pm, 4:00 pm, and 7:00 pm); after solid and water fasting, the birds were slaughtered to assess the absolute weight of the organs. The time of day influenced ($p < 0.05$) the physiological parameters RR and AST, and CT showed a significant effect ($p < 0.05$) of increasing levels of digestible threonine. The total weight of the pancreas, proventriculus and lung showed a significant effect ($p < 0.05$) of the increase in the levels of digestible threonine. The respiratory rate is affected by the levels of threonine in the diet. The 0.687% level promoted hypertrophy of the pancreas, proventriculus and lung, promoting more significant activity of these organs.

Keywords: amino acids; environment; ideal protein; metabolism.

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Introduction

Decades after the industrial growth of broiler production, research on low crude protein amino acids continued, but it was widely accepted that dietary inclusion of amino acids other than sulfur-containing amino acids and lysine would not economically sustain production in practice (Costa, Silva, Goulart, Nogueira, Sá, 2014). In the late 20th century, the adoption of the third food-grade amino acid, namely L-threonine (Thr), was aided by advances in optimal protein formulation tools and the expression of amino acids in dietary formulation on a digestible basis (Chalova, Kim, Patterson, Ricke, Kim, 2016).

Due to the precocity and high peak production of commercial layers, it is necessary to reassess such animals' nutritional needs to guarantee maximum performance. However, these birds require the supply of nutrients in adequate amounts, including amino acids, to produce eggs at levels compatible with their genetic potency (Pastore et al., 2016; Chrystal et al., 2020).

The formulation of diets based on total amino acids can lead to deficiencies and/or excesses in their supply (Kidd, Maynard, & Mullenix, 2021). As a measure to circumvent such a problem, the concept of ideal protein arises, which allows formulations of diets without excesses and deficiencies of essential amino acids (Schmidt et al., 2011).

Food is the most expensive part of production costs, accounting for approximately 70% of total costs. Protein and energy are the most representative parts of these costs because they are expensive (Star, Rovers, Corrernt, & Klis, 2021). It is essential to know the performance of strains of egg-producing birds subjected to different levels of these nutrients, trying to determine the most suitable levels to minimize production costs (Ribeiro, Matos Júnior, Queiroz, Lara, & Baião, 2013). On the other hand, synthetic amino acids' availability allows nutritionists to formulate poultry feeds based on the crude protein requirement adjusted with respect to the amino acid requirements (Viola, Ribeiro, Beretta Neto, & Kessler, 2008).

Due to the commercial availability of synthetic amino acids at compatible prices, there is currently an increasing practice of incorporating these amino acids in diets, allowing formulations of minimum cost with crude protein levels lower than those recommended in the tables of nutritional requirements, but meeting the requirements in essential amino acids (Star et al., 2021). Threonine is an essential amino acid for birds, considered the third limiting amino acid in diets for laying hens (Pastore et al., 2016). The ratio of 76% digestible lysine for light laying hens with 51g egg mass is recommended (Rostagno et al., 2011). In the literature, reports have been observed in several studies which tested the reduction of protein levels in diets. However, these studies have focused mainly on the study of nutrition on animal performance, thus not focusing on other aspects such as the interaction between nutrition and animal physiology.

Thus, it is of great importance and necessary to know how to modify the diets given to animals according to the climate, their direct effects on physiology, and their direct effects on the size of birds' internal organs. The objective of this study was to evaluate the effect of threonine:digestible lysine ratio in the diet on the physiological parameters and organ weights of light layers.

Material and methods

Experiment location and animals

The experiment was conducted in the Poultry sector of the Federal University of Paraíba – Center for Agricultural Sciences – Campus II, located in Areia, Paraíba state, Brazil. The Areia city is located in the Brejo Paraibano microregion, North Region of the State of Paraíba at 6°57'48" south latitude, 35°41'30" west longitude, at an altitude of 618m above the sea level. According to Köppen's classification, the region has an AS-type climate, corresponding to the hot and humid climate, with autumn-winter rains.

The experiment was carried out in the rainy season of the year, and the animals were kept in clay tile sheds, open on the sides with curtain protection and without an air conditioning system.

Two hundred and ten 47-week-old Dekalb White laying hens were distributed according to a completely randomized experimental design, in five treatments, seven repetitions, totaling 35 plots of eight birds each. The layers were housed in wire cages with dimensions of 24 cm x 37 cm x 41 cm, equipped with feeders and semi-automatic drinkers. The experimental period was 10 weeks, totaling 62 days and more eight days for the animals to adapt.

Diets

The feed was supplied twice a day, in the morning (5:00 am) and in the afternoon (5:00 pm), to guarantee free consumption to the animals, and water was supplied to the animals *ad libitum*.

The experimental diets were isocaloric and consisted of a basal diet formulated based on corn and soybean meal, supplemented with industrial amino acids L-lysine, DL-methionine, L-tryptophan, L-arginine, L-isoleucine, and L-valine, providing increasing levels of these amino acids so that the relationships remain constant, based on the recommendations of Rostagno et al. (2005), with digestible threonine:digestible lysine ratio, that is, 75% of this ratio, so that the levels of digestible threonine used were 0.507, 0.552, 0.597, 0.642 and 0.687% (Table 1). Feed consumption (g bird^{-1} day^{-1}) of birds fed different digestible threonine levels in the diet was analyzed weekly (Table 2).

Physiological parameters

The parameters studied were respiratory rate (RR), cloacal temperature (CT), and average surface temperature (AST) throughout the experiment. Each of these variables was evaluated at different times (7:00 am, 10:00 am, 1:00 pm, 4:00 pm, and 7:00 pm), two hours after the feed supply, to avoid the caloric interference increase. Two birds from each plot were used, which were marked and identified.

The respiratory rate (mov min^{-1}) was obtained through visual evaluation, considering the number of times the birds inhaled air, for 20 seconds, and later the value obtained was multiplied by three. For cloacal temperature (°C), a digital veterinary clinical thermometer was inserted about 2 cm into the birds' cloaca kept for 2 minutes on average or until the temperature stabilized, and read. For AST, a laser-sight infrared thermometer (Model AK 32), with operation temperature ranging from -10 to 50°C, was used to measure the temperatures of the head, crest, back, wing and feet; then, the mean AST was calculated (Rodrigues, Furtado, Costa, Nascimento, & Cardoso, 2016).

Table 1. Food and chemical composition of experimental feed.

Ingredient		Threonine levels (%)				
		0.507	0.552	0.597	0.642	0.687
Corn		67.80	67.56	67.31	67.07	66.83
Soybean meal 45%		18.23	18.26	18.30	18.34	18.38
Limestone		9.30	9.30	9.30	9.30	9.30
Dicalcium phosphate		1.55	1.55	1.55	1.55	1.55
Soy oil		2.03	1.96	1.88	1.81	1.74
Salt		0.52	0.52	0.52	0.52	0.52
DL-Methionine		0.19	0.24	0.30	0.35	0.41
L-Lysine		0.07	0.13	0.19	0.24	0.30
L-Threonine		0.03	0.08	0.12	0.17	0.21
L-Isoleucine		0.03	0.08	0.13	0.19	0.23
L-Valine		0.02	0.07	0.13	0.18	0.23
L-Tryptophan		0.01	0.02	0.03	0.05	0.06
Complete premix[12]		0.10	0.10	0.10	0.10	0.10
Hill (70%)		0.07	0.07	0.07	0.07	0.07
BHT[3]		0.01	0.01	0.01	0.01	0.01
Calculated composition						
Crude protein %	16.50	14.13	14.35	14.57	14.79	15.00
Calcium %	4.02	4.02	4.02	4.02	4.02	4.02
Available Phosphorus %	0.37	0.37	0.37	0.37	0.37	0.37
E. Met. Poultry kcal kg^{-1}	2900	2900	2900	2900	2900	2900
Dig. Ile Birds %	0.66	0.56	0.61	0.66	0.71	0.76
Dig. Lys Birds %	0.79	0.67	0.73	0.79	0.85	0.91
Dig. Met Birds %	0.39	0.40	0.46	0.51	0.57	0.62
Dig. M + C Birds %	0.72	0.61	0.67	0.72	0.78	0.83
Dig. Thr Birds %	0.52	0.50	0.55	0.59	0.64	0.68
Dig. Trp Birds %	0.18	0.15	0.16	0.18	0.19	0.21
Dig. Val Birds %	0.71	0.60	0.66	0.71	0.77	0.82
Sodium %	0.22	0.22	0.22	0.22	0.22	0.22
Chlorine %	0.20	0.35	0.35	0.35	0.35	0.35
Potassium %	0.58	0.58	0.58	0.58	0.58	0.58
Threonine / Lysine	66	75	75	75	75	75
Methionine / Lysine	50	60	63	65	67	68
Met + Cyst / Lysine	91	91	91	91	91	91
Tryptophan / Lysine	23	23	23	23	23	23
Isoleucine / Lysine	83	83	83	83	84	83
Valine / Lysine	90	90	90	90	90	90

Premix Vitamínico1 Composition / kg of product: Vit. A - 12,000,000 FTU (IU); Vit. D3 - 3,600,000 FTU (IU); Vit. B1 - 2.5 g; Vit. B2 - 8 g; Vit. B6 - 3 g; Pantothenic acid - 12 g; Biotin - 0.2 g; Vit. K - 3 g; Folic acid - 3.5 g; Nicotinic acid - 40 g; Vit. B12 - 20 mg; Se - 0.13 g; Vehicle q.s. - 1000 g. Premix Mineral2 Composition / kg of product: Mn - 160 g; Fe - 100 g; Zn - 100 g; Cu - 20 g; Co - 2 g; I - 2 g. Excipient q.s. - 1,000 g. BHT[3]= Butyl-Hydroxy-Toluene (antioxidant).

Table 2. Average feed consumption (g bird^{-1} day^{-1}) of birds receiving diets with different levels of digestible threonine.

Digestible threonine levels (%)	Feed consumption g bird^{-1} day^{-1}
0.507	102.64
0.552	103.29
0.597	102.31
0.642	102.34
0.687	102.73

Climatic environment

Meteorological variables inside the facilities were recorded during the experiment; air temperature (Tair, °C) and relative air humidity (RH, %) were measured every 3 h for 24 h, using HT-500 data loggers (Instrutherm Instrumentos de Medição Ltda, SP). The devices were installed in the center of each facility and, based on the obtained data, the temperature-humidity index (THI) was calculated through the equation proposed by Buffigton et al. (1981).

Slaughter

At the end of the experiment, ten birds from each treatment were slaughtered to assess internal organs' weight. The slaughter was carried out on the same day, in the morning, after the animals had gone through a 12-hour fasting period. Before slaughter and after the fasting period, all layers were weighed individually to obtain live weight at slaughter. At the slaughter time, the layers were hung in the slaughter funnel, and immediately afterward, the animals were exsanguinated by hand cutting at the jugular level. Then, after draining the blood for three minutes, the animal's weight was obtained without the blood and feathers. Then, the animals were subjected to skinning, evisceration, and weighing of the following organs: pancreas (g), proventriculus (g), gizzard (g), liver (g), intestine (g), lung (g), heart (g), and oviduct (g). The intestine length was also measured.

Statistical analysis

The physiological indices were evaluated within a 5x5 factorial arrangement (five hours and five levels of threonine), using Tukey test at 5% probability level by the PROC GLM procedure; for the effect of diet, polynomial regression analysis was performed by the PROC REG procedure using the program Statistical Analyses System (SAS, 2001). The absolute weight of the organs was analyzed as a covariate through statistical analysis.

Results and discussion

It is observed that the time of day influenced ($p < 0.05$) the physiological parameters RR and AST. However, BGTHI was not influenced by the time of day ($p > 0.05$) (Table 3).

Table 3. Average respiratory rate (RR), cloacal temperature (CT), and average surface temperature (AST) of light laying hens and BGTHI as a function of time.

Hours	RR (mov. min^{-1})	CT (°C)	AST (°C)	BGTHI
07:00	43.3b	40.5[a]	32.3b	73.1
10:00	49.1[a]	40.6[a]	33.9a	75.9
13:00	49.5[a]	41.0a	34.3a	79.9
16:00	50.6[a]	40.7[a]	32.9b	76.9
19:00	45.0b	40.6[a]	32.2b	72.7
Coefficient of variation (%)	25.6	3.0	16.5	-

Averages followed by the same letter in the column do not differ significantly for the same parameter by the Tukey test at 5% probability level.

According to Baêta and Souza (2010), the BGTHI between 74 and 78 is within the stress zone for the birds, and under this condition there is the most significant discomfort for the birds at 1 pm. Considering that the installations are made of clay tile and open with curtain handling, RR had the highest averages between 10 am and 4 pm, while at 7 am and 7 pm, the values were lower and statistically equal. RR showed more significant activity from 10 am to 4 pm, concomitantly with BGTHI ranging from 75.9 to 79.9 (Table 3). The high respiratory activity can be an efficient strategy for short-term heat loss; if this activity is prolonged due to heat stress, it results in severe problems for the animals, and consequently, in food intake (Rodrigues et al., 2016). According to Rodrigues et al. (2016), under heat stress conditions, birds tend to increase their respiratory rate by up to ten times their usual rhythm in response to thermal discomfort, indicating the use of heat exchange mechanisms.

The obtained averages of CT did not show any difference between the evaluated times, as this is the last variable affected by the increase in the ambient temperature. In the present study, the CT varied between 40.5 and 41.0°C; as it remained intermediate, the birds managed to maintain homeothermy and remained within the normal temperature range for birds (Castilho et al., 2015).

The respiratory rate was significantly affected ($p < 0.05$) by the increasing levels of digestible threonine, while CT and AST were not affected ($p > 0.05$) (Table 4). The recommended level of the threonine-to-digestible lysine ratio for light laying hens is 76%; below this level, there is deficiency of such nutrient (Rostagno et al., 2011; Hy-Line do Brasil, 2015). Deficiency of this amino acid results in growth retardation, perosis and liver problems due to the difficulty in mobilizing fat for circulation because, under VLDL deficiency, the organism seeks replacement from body protein through protein degradation, which causes a more significant internal heat input in the animal, resulting in a more significant respiratory activity (Sakomura, 2014). The RR adjustments were sufficient to keep CT and AST unchanged.

Table 4. Means of respiratory rate (RR, movement/min), cloacal temperature (CT, °C) and average surface temperature (AST, °C) of light laying hens as a function of threonine levels (%).

Variables	Digestible threonine levels (%)					Equation (R^2)
	0.507	0.552	0.597	0.642	0.687	
RR	49.0ab	50.0a	49.0ab	47.0b	46.0b	$y = 50.85-0.91x$ ($R^2 = 0.75$)
CT	40.8	40.6	40.7	40.9	40.7	not significant
AST	33.5	33.6	33.7	33.3	33.2	not significant

Averages followed by the same letter in the row do not differ significantly for the same parameter by the Tukey test at 5% probability level.

The AST had higher values at 10 am and 1 pm. At other times the values were lower and statistically equal. The highest AST average was observed between 10:00 am and 1:00 pm, with 33.9 and 34.3°C, respectively, concomitant with the highest BGTHI average at 1:00 pm, with a value of approximately 80. For Vieira et al. (2021) and Rodrigues et al. (2021), the surface temperature depends directly on the environment's conditions: sudden changes in the meteorological variables in the place where the animal is found results in changes in the surface temperature. Similar results were obtained by Nascimento, Pereira, Nââs, and Rodrigues (2011), who found a decrease in surface temperature with increasing age in male broilers raised under natural conditions of temperature and humidity. At 23 days of age, values between 34.3 and 37.4°C and between 34.9 and 37.5°C were observed at 27 days. At 32, 36 and 41 days, a decrease in surface temperature was observed, with records between 31.9 and 35.2°C, 33.4 and 35.3°C, and 32.9 and 34.9°C, respectively.

As birds suffer heat stress, climatic factors influence the animals' physiology, causing them to dissipate heat through physiological mechanisms, one of which is peripheral vasodilation, increasing blood flow to the skin and limbs, consequently resulting in higher surface temperature (Costa, Saraiva, Costa, Santos, 2012). According to Ruzal et al. (2011), part of the physiological responses that explain the direct relationship between ambient temperature and the surface temperature of birds may be related to the redistribution of blood flow in the body, since exposure to heat causes peripheral vasodilation. Camerini, Silva, Nascimento, Oliveira, and Souza (2016), who evaluated the surface temperature of commercial laying birds raised under different climatic conditions (20, 26 and 32°C), observed that body, head and leg temperatures increased with increasing air temperature, and the head temperature values were higher when compared to the body and leg temperatures.

The balance of amino acids has a direct effect on the thermal balance of the organism; depending on such balance, a series of metabolic reactions are triggered, which culminates in the release of internal heat, and the primary physiological process involved in the release of extra heat occurs through respiratory activity (Costa et al., 2012).

The total weights of pancreas, proventriculus, and lung were significantly affected ($p < 0.05$) by the increasing levels of digestible threonine. At the same time, for the gizzard, liver, intestine, heart, and oviduct, there was no significant effect ($p > 0.05$) (Table 5).

Table 5. The average absolute weight of organs (g) in light hens receiving diets with different levels of digestible threonine.

Organs	Digestible threonine levels (%)					Equation (R^2)
	0.507	0.552	0.597	0.642	0.687	
Pancreas (g)	2.79	3.41	3.78	3.15	4.55	$y = 2.55+0.32x$ ($R^2 = 0.58$)
Proventriculus (g)	6.02	7.04	7.35	6.59	7.75	$y = 6.04+0.30x$ ($R^2 = 0.50$)
Gizzard (g)	27.44	27.97	29.53	31.09	29.13	not significant
Liver (g)	28.20	29.63	28.73	28.65	27.55	not significant
Intestine (g)	42.04	39.58	43.96	39.59	43.48	not significant
Small intestine (cm)	140.6	129.4	136.6	130.1	135.5	not significant
Lung (g)	5.74	6.25	6.70	6.08	7.01	$y = 5.64 + 0.23x$ ($R^2 = 0.55$)
Heart (g)	6.87	7.43	7.81	6.98	8.03	not significant
Oviduct (g)	63.99	59.13	62.97	54.58	60.26	not significant

Different letters in the row differ by Tukey test at 5% probability level.

The pancreas' total weight (g) showed a linear effect with the addition of digestible threonine in the diet, reaching 4.55g in birds supplemented with 0.687% digestible threonine. This result occurs due to the more significant supply of nutrients to be digested, demanding greater pancreatic activity.

The greater total weight of proventriculus is perceived in birds (7.75g) that received supplementation with a higher digestible threonine level in the diet 0.687%. Costa et al. (2012), when working with the absolute weight of organs of laying hens receiving different levels of tryptophan, observed a gradual increase in the proventriculus due to the increased supplementation of the amino acid, reaching such an organ weighing 9.51g, when receiving 0.215 % of tryptophan.

The highest absolute lung weight (7.01g) was observed for birds supplemented with the highest digestible threonine level in the diet (0.687%). There is a reduction in the absolute weight of all organs of light laying hens receiving lower levels of the amino acid. Girôtto et al. (2013), working with amino acid supplementation in diets for piglets weaned at 21 days of age, observed that there was no reduction in the size of all absolute organs of pigs due to the reduction of protein in the diet.

Conclusion

Respiratory rate, cloacal temperature, and average surface temperature vary throughout the day depending on climatic variables. Respiratory rate is affected by dietary threonine levels. The 0.687% level promoted hypertrophy of the pancreas, proventriculus, and lung, leading to greater activity of these organs.

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