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VITAMIN C AND SELENIUM ON PHYSIOLOGICAL VARIABLES AND DIET METABOLIZABILITY IN BROILERS

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Abstract: This study was carried out to evaluate the effect of vitamin C and selenium on nutrient metabolizability, nitrogen balance and utilization efficiency. 140 chickens were used for the metabolism assay. In the experimental trial, a 2 x 3 + 1 factorial scheme was used, with two levels of vitamin C supplementation (150 and 300 mg of coated ascorbic acid/kg of feed), associated with three levels of selenium (0.2; 0.4 and 0.6 mg of selenium yeast/kg of ration) in addition to a control treatment (without vitamin C and selenium supplementation) totaling seven treatments and five replications, with animals submitted to a natural condition of high temperature. Supplementation of 300 mg of vitamin C associated with 0.6 mg of selenium per kilogram in diets for broilers, under environmental conditions above the thermoneutrality zone in the evaluated phase, provides better availability of apparent metabolizable energy when corrected for nitrogen balance, and does not present a positive effect for the other variables studied in relation to the control group.

Keywords: Heat stress, antioxidant, nitrogen balance, liver enzymes.

INTRODUCTION

The production of broiler chicken has high production rates due to the technologies adopted in the most differentiated extracts of exploitation of the poultry activity. However, performance can be impaired when birds are subjected to temperatures outside the thermoneutrality zone, and this climatic element can act as an inducer of heat stress (WAN et al., 2018), represented by the biological and behavioral reaction of the animal organism. to stimuli, which upset their normal physiological balance.

Heat stress alters physiological metabolism, causes disorder in the endocrine system (LARA; ROSTAGNO, 2013), impairs immune function (HOSSEINI-VASHAN; GOLIAN; YAGHOBFAR, 2015) and induces oxidative damage (HABIBIAN; GHAZI; MOEINI, 2015). Thus, the search for alternatives to improve the productive indicators of chickens in high temperature environments, mobilizes Brazilian producers and technicians, given the tropical conditions prevailing in our territory. In this context, Liao et al. (2018) highlight that the adoption of nutritional strategies such as vitamin and mineral supplementation to meet the needs of chickens during heat stress can minimize the deleterious effects caused by high temperatures.

Vitamin C is essential in the maintenance and efficiency of homeostasis, as it is a potent electron-donating antioxidant, used to reduce the adverse effects of heat stress, such as lipid peroxidation (PISOSCHI; POP, 2015). Under homeostasis conditions, chickens synthesize ascorbic acid in the kidneys, being able to meet their requirements (MAURICE et al., 2002). However, this production becomes insufficient to meet biological demands in adverse environmental conditions, such as high temperature.

Selenium is an essential mineral that plays an important role in the antioxidant system through selenoproteins, which act in the maintenance of redox balance and antioxidant defenses (SURAI; FISININ, 2014). Selenium stands out in association as an essential part of selenoprotein glutathione peroxidase (GSH-Px), to catalyze the reduction of hydrogen peroxide and lipid peroxides to less harmful hydroxides (DALIA et al., 2018).

Given these considerations, the objective was to evaluate the effect of vitamin C and selenium supplementation in diets for broilers on the metabolizability of nutrients, balance and efficiency of nitrogen use, body temperature and blood biochemistry.

MATERIAL AND METHODS

The experiment carried out aimed to evaluate the metabolizability of nutrients, nitrogen balance and nitrogen utilization efficiency in the diets. The experiment was carried out in the poultry sector shed of the Animal Science Department (DZO), of the Agricultural Sciences Center (CCA), of the Federal University of Piauí (UFPI) in Teresina - Piauí. All procedures inherent to the research performed were submitted to and approved by the ethics committee on the use of animals at UFPI under protocol No. 355/17.

The municipality of Teresina is located at latitude 05º 05'21" south and longitude 42º 48' 07" west, with an altitude of 74.4 meters, with average annual minimum and maximum temperatures of 22.2 and 34ºC, respectively (SILVA et al, 2015).

METABOLIZABILITY OF DIETARY NUTRIENTS

A total of 140 male broilers, from the Ross 308 AP strain, were used in the period from 22 to 29 days of age. Until the 21st day of life, the birds were kept in a shed, receiving a diet formulated to meet the nutritional requirements, according to the feeding programs recommended by Rostagno et al. (2017). On the 22nd day of age, the birds were weighed and distributed in metabolic cages, starting the period of adaptation to the ration and the facilities lasting four days and later, the collection of excreta began, lasting four days, totaling eight days. of the experiment.

The design adopted was completely randomized, in a 2 x 3 + 1 factorial scheme, with two levels of vitamin C (150 and 300 mg of coated ascorbic acid/kg of feed), associated with three levels of selenium (0.2, 0.4 and 0.6 mg of selenium yeast/kg of feed) and a control diet (without vitamin C and selenium supplementation, but with baseline levels of 0.492 mg of selenium in phase 22 to 33, in accordance with the nutritional composition of the ingredients and the premix), totaling seven treatments and five repetitions. The experimental unit was represented by four birds/cage, totaling 20 birds per treatment.

The chickens received isoprotein and isoenergetic experimental diets, based on corn and soybean meal, formulated to meet the nutritional requirements in the growth phase (22 to 33 days of age) (Table 1), as recommended by Rostagno et al. (2017).

The rations were fed ad libitum and weighed at the beginning and end of the collection period, to quantify the consumption per experimental unit. In each treatment, the ferric oxide marker was added to the first meals of the first and last day of collection. Thus, the definition of the beginning and end of the collection period was based on the appearance of the marked excreta, so that the unmarked excreta in the first collection, and those marked in the last collection, were discarded.

The birds received clean water ad libitum, changed twice a day. Environmental temperature monitoring was performed using thermohygrometers, with records made daily at 8:00 and 16:00. The lighting program adopted was continuous (24 hours of natural + artificial light), using fluorescent lamps.

The technique used was the total collection of excreta in each cage, performed twice a day, at 12-hour intervals. After collection, the excreta were placed in plastic bags, identified, weighed and stored in a freezer for laboratory analysis.

At the end of the collection period, all the excreta from the same experimental unit was thawed at room temperature and mixed uniformly to obtain a sample. After pre-drying, in an oven with forced air circulation, for 72 hours at 60 ± 5ºC, the excreta were ground in a knife mill, as were the test diets. The samples were analyzed for dry matter, total nitrogen and gross energy and mineral matter contents according to the procedures of Silva and Queiroz (2002).

From the data of feed consumption, excreta production and laboratory analysis of diets and excreta, the metabolizability coefficients were calculated: a) of dry matter (DMMS), b) of crude protein (CMPB), c) of energy gross; d) nitrogen balance (BN); e) nitrogen utilization efficiency (EUN) and f) apparent metabolizable energy corrected for nitrogen balance (EMAn). To calculate the metabolizability coefficient, the following equation proposed by SAS et al. (1965).

MC = [(nutrient ingested – nutrient excreted) x 100] / nutrient ingested;

BN = N ingested – N excreted;

EUN = [N ingested – N excreted)] x 100] / N ingested;

EMAn = [EB ingested - (EB excreted + 8.22 x BN)] / MS ingested.

For the experimental test, the mean and standard deviation of the environmental variables were calculated. The other parameters were submitted to the evaluation of homogeneity and normality, and the identified outliers were removed. Subsequently, the data were subjected to analysis of variance, and when significant, vitamin C supplementation levels were compared by Tukey's tests, and for selenium levels, polynomial regression analysis was used. When comparing each treatment with the control diet, the Dunnett test was applied, according to the statistical procedures of the PROC GLM of the Statistical Analysis System software. $\alpha = 0.05$ was considered.

RESULTS AND DISCUSSION

The average values of maximum and minimum temperatures remained above the comfort zone during the experimental test, according to Abreu and Abreu (2011) (Table 3).

In environments considered thermoneutral, the intestine of broilers has the ability to efficiently digest and absorb nutrients via specific receptors. However, heat heat stress negatively influences nutrient consumption and metabolism, reducing the concentration of micronutrients such as vitamins A, E and C, and Se (RENAUDEAU et al., 2011), it also impairs the integrity of the intestinal wall and causes immune system disorders. All these factors cause poor performance, greater susceptibility to diseases and higher mortality in chickens (VARASTEH et al., 2015).

According to Sahin et al. (2009), broilers subjected to heat stress have increased mineral excretion and reduced concentrations of antioxidant vitamins (Vitamins E, C, and A) and minerals (e.g., Se, Zn, and Cr) in the serum and liver of birds..

Regarding metabolizability, no interaction was observed between the levels of vitamin C and selenium for the coefficients of metabolizability of dry matter (DM) and crude protein (CP) ($P>0.05$), with the exception of the coefficient of gross energy (EB) ($P<0.05$) (Table 4). The isolated levels of vitamin C and selenium did not influence the metabolizability coefficients of dry matter and crude protein ($P>0.05$). When comparing the control treatment with the others, no difference was observed ($P>0.05$). In the decomposition of the interaction, it was observed that the level of 0.6 mg of selenium/kg of the diet, associated with the level of 300 mg of vitamin C ($P<0.05$), provided a higher value for the energy metabolizability coefficient., while at the levels of 0.2 and 0.4 mg of selenium/kg of feed, there was no difference between the two levels of vitamin C ($P>0.05$). For this same variable, there was a quadratic effect of selenium levels associated with 300 mg of vitamin C/kg in the diet, represented by the equation $Y = 43.75x^2 - 29.9x + 76.44$ ($R^2 = 1$), with lower metabolic efficiency observed at the

Ingredients (%)	Vitamin C Levels/Selenium Levels (mg/kg)						
	0/0	150/0.2	150/0.4	150/0.6	300/0.2	300/0.4	300/0.6
Corn (7.86% CP)	57,348	57,297	57,277	57,257	57,265	57,244	57,224
Soybean meal (46% CP)	34,491	34,500	34,503	34,507	34,505	34,509	34,512
Vegetable oil	4,467	4,484	4,491	4,498	4,495	4,502	4,509
Dicalcium phosphate	1,444	1,444	1,444	1,444	1,444	1,444	1,444
calcitic limestone	0.722	0.721	0.721	0.721	0.721	0.721	0.721
Common salt (NaCl)	0.492	0.492	0.492	0.492	0.492	0.492	0.492
L-Lysine-HCl (79%)	0.036	0.036	0.036	0.036	0.036	0.036	0.036
DL-Methionine (98%)	0.001	0.001	0.001	0.001	0.001	0.001	0.001
[1] Premix	1,000	1,000	1,000	1,000	1,000	1,000	1,000
[2] Selenium	0.000	0.010	0.020	0.030	0.010	0.020	0.030
[3] Vitamin C	0.000	0.015	0.015	0.015	0.031	0.031	0.031
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Crude protein (%)	20,580	20,580	20,580	20,580	20,580	20,580	20,580
ME (kcal/kg)	3,150	3,150	3,150	3,150	3,150	3,150	3,150
Digestible lysine (%)	1,124	1,124	1,124	1,124	1,124	1,124	1,124
Digestible methionine (%)	0.548	0.548	0.548	0.548	0.548	0.548	0.548
Methionine + digestible cystine (%)	0.832	0.832	0.832	0.832	0.832	0.832	0.832
Digestible threonine (%)	0.708	0.708	0.708	0.708	0.708	0.708	0.708
Digestible tryptophan (%)	0.238	0.238	0.238	0.238	0.238	0.238	0.238
Calcium (%)	0.758	0.758	0.758	0.758	0.758	0.758	0.758
Available phosphorus (%)	0.374	0.374	0.374	0.374	0.374	0.374	0.374
Sodium (%)	0.208	0.208	0.208	0.208	0.208	0.208	0.208
Selenium (mg/kg)	0.492	0.692	0.892	1,092	0.692	0.892	1,092
Vitamin C (mg/kg)	0.000	150,000	150,000	150,000	300,000	300,000	300,000

[1] Guarantee levels per kg of product: iron 4,000.00 mg; copper 1000.00 mg; manganese 7,000.00 mg; zinc 6,000.00 mg; iodine 100.00 mg; selenium 30.00 mg; vitamin A 700,000.00 IU; vitamin D3 160,000.00 IU; vitamin E 1,400.00 IU; vitamin K3 180.00 mg; vitamin B1 150.40 mg; vitamin B2 500.00 mg; niacin 3,000.00 mg; pantothenic acid 1160.00 mg; vitamin B6 240.00 mg; folic acid 80.00 mg; biotin 4.00 mg; vitamin B12 1,000.00 mcg; choline 37.00 g; lysine 90.00 g; methionine 265.00 g; salinomycin 6,600.00; enramycin 1000.00 mg; [2] Guarantee levels: Selenium yeast (min.): 2,000.00 mg/kg; [3] Levels of Warranty: Coated Vitamin C – Ascorbic Acid 97.68%.

Table 1. Percentage composition and nutritional content of experimental diets for broilers from 22 to 33 days of age

experiment 1			
days of life	Temperature (°C)		Relative humidity (%)
	Minimum	Maximum	
22 - 28	23.86 ± 0.90	32.84± 3.11	66.30 ± 13.28

[1] Average values. ITGU - Globe Temperature and Humidity Index.

Table 3 . Environmental conditions observed during the experimental period [1]

0.34 mg selenium level. When comparing the control treatment with the other treatments tested for these parameters, no significant difference was observed (P>0.05).

The need to maintain the thermal comfort of broilers aims to ensure that the smallest fraction of food energy is used to maintain body temperature, and that body production benefits the most (DALÓLIO et al., 2015). However, the average temperature recorded shows that the animals were subjected to a temperature above the thermoneutrality zone, which may have influenced a greater production of free radicals, leading to oxidative stress and a reduction in the amount of energy for production (EL -TARABANY, 2015).

Thus, considering that vitamin C, because it has an antioxidant function (TORKI; ZANGENEH; HABIBIAN, 2014), favors the maintenance of the intestinal mucosa epithelium and the vessel wall (ATTIA et al., 2016) and promotes the reduction of tissue degradation (FERNANDES et al., 2013), acting in association with selenium, which also has antioxidant characteristics in the body (KHOSO, 2016). In this scenario, it was expected that the supplementation of diets with these nutrients would present better use in the coefficients of metabolizability of dry matter, crude protein and crude energy, in relation to the control group. However, in the present work, there was no difference between the control treatment and the others for the metabolizability coefficients of dry matter, protein and energy.

The negative effect of high temperature is well portrayed by Souza et al. (2016), who, when evaluating the digestibility coefficients of dry matter, crude protein and crude energy in the period from 39 to 42 days of age for broilers in different environmental conditions (22º and 32º C), observed better coefficients for the variables evaluated in animals submitted to a temperature of 22º C.

By supplementing 200 mg of ascorbic acid/kg in the diet for laying hens subjected to heat stress, Attia et al. (2016) found better crude protein digestibility and equivalent values for dry matter digestibility compared to the control group, thus proving the efficiency of vitamin C in crude protein digestibility in high temperature environments, which implies a better use of nutrients. amino acids (ABU-DIEYEH, 2006). However, these results were not corroborated in the present study.

It was verified that there was no interaction (P>0.05) between the levels of vitamin C and selenium tested, for the nitrogen balance (BN), however this effect was evidenced (P<0.05) for efficiency of use of nitrogen (EUN) and for apparent metabolizable energy corrected for nitrogen balance (EMAn) (P<0.05) (Table 5).

Regarding nitrogen balance, there was no influence of vitamin C or selenium levels (P>0.05). And in the comparison of the control treatment with the others, no difference was observed for nitrogen balance and nitrogen use efficiency (P>0.05).

Regarding EUN, a linear effect (P<0.05) was observed for the levels of selenium associated with 150 mg of vitamin C/kg of the diet, according to the equation $y = 65.17 - 6.65x$ ($R^2 = 0.89$), so that, as selenium levels increased, the EUN decreased. On the other hand, at the level of 300 mg of vitamin C/kg of feed, there was no effect of selenium levels (P>0.05), and at each selenium level, there was no difference between vitamin C levels (P >0.05).

For Temim et al. (1999), exposure of chicken to high temperatures negatively affects protein metabolism, decreasing nitrogen consumption, compromising its efficiency. However, strategies involving dietary supplementation with ascorbic acid and selenium can mitigate these negative effects.

Control	vit C mg/kg	Selenium (mg/kg)			Average [1]	CV (%)	P [2] value	
		0.2	0.4	0.6			L	Q
Dry matter metabolizability coefficient (%)								
69.85	150	69.97	69.49	69.25	69.57 to	1.46	0.7195	0.8689
	300	69.31	69.76	69.70	69.59 to			
	Average	69.64	69.62	69.47				
Crude protein metabolizability coefficient (%)								
65.24	150	66.24	64.17	63.80	64.74 to	2.11	0.1049	0.6591
	300	63.77	65.28	64.13	64.39 to			
	Average	65.00	64.72	63.97				
Gross energy metabolizability coefficient (%)								
72.85	150	73.20 to	71.90 to	72.09 b	72.40	1.60	0.1419	0.2437
	300	72.21 to	71.48 to	74.25 to	72.65		0.0149	0.0158
	Average	72.70	71.69	73.17				

[1] Means followed by the same lowercase letter, in the column, for the same variable, do not differ from each other by Tukey's test (P>0.05). [2] L, Q: linear and quadratic order effects, respectively, related to dietary selenium inclusion.

Table 4. Metabolizability coefficient of dry matter, crude protein, crude energy, of diets, supplemented with vitamin C and selenium, for broilers from 22 to 42 days

Control	vit C mg/kg	Selenium (mg/kg)			Average [1]	CV (%)	P [2] value	
		0.2	0.4	0.6			L	Q
Nitrogen balance (g/day)								
2.09	150	2.14	2.08	2.09	2.10 to	5.19	0.5226	0.9634
	300	2.03	2.07	2.02	2.04 to			
	Average	2.09	2.07	2.05				
Nitrogen utilization efficiency (%)								
63.15	150	64.10 to	61.99 to	61.44 to	62.51	2.47	0.0091	0.3143
	300	61.51 to	63.12 to	61.93 to	62.19		0.7407	0.2126
	Average	62.81	62.55	61.68				
EMAn (kcal/kg)								
3126.21	150	3149.82 to	2984.55 to *	3079.65 b	3071.34	1.63	0.0387	0.0003
	300	3134.43 to	2910.27 to *	3366.80 to *	3137.17		<.0001	<.0001
	Average	3142.12	2947.41	3223.23				

[1] Means followed by an asterisk differ from the control treatment by Dunnett's test (P<0.05). [2] Means followed by the same lowercase letter, in the column, for the same variable, do not differ from each other by Tukey's test (P>0.05). [3] L, Q: linear and quadratic order effects, respectively, related to dietary selenium inclusion.

Table 5 . Nitrogen balance (BN), nitrogen utilization efficiency (EUN) and apparent metabolizable energy corrected by nitrogen balance (EMAn) of diets supplemented with vitamin C and selenium for broilers in the final phase

For the AMEn variable, it was observed that at the level of 0.6 mg of selenium/kg in the diet, the highest value occurred when associated with 300 mg compared to the level of 150 mg of vitamin C (<0.05). However, at the levels of 0.2 and 0.4 mg of selenium, there was no effect of vitamin C (P>0.05). Also, there was a quadratic effect (P<0.05) of selenium levels associated with 150 and 300 mg of vitamin C/kg in the diet, represented, respectively, by the equations $Y=3254.6x^2 - 2779.1x + 3575.5$ ($R^2 = 1$), with the lowest value observed at the level of 0.42 mg of selenium and $Y = 8508.6x^2 - 6226x + 4039.3$ ($R^2 = 1$), with the lowest energy level observed with 0.36 mg of selenium.

In comparing the control treatment with the other test treatments, the AMEn value of the control diet showed a higher value compared to supplementation of 150 and 300 mg of vitamin C/kg of the diet associated with 0.4 mg of selenium, and a lower value, when compared to the highest level of association of the test nutrients. So,possibly, this greater association may have contributed to a better supply of energy to body development at times when these animals were subjected to temperatures outside the thermal comfort zone.

CONCLUSION

Supplementation of 300 mg of vitamin C associated with 0.6 mg of selenium per kilogram in diets for broilers in environmental conditions above the thermoneutrality zone for the 22 to 42 day-old phase improves the availability of balance-corrected apparent metabolizable energy of nitrogen.

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