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Original Articles

Organic zinc and vitamin E supplementation for broiler chickens under natural heat stress conditions

Suplementação de zinco orgânico e vitamina E para frangos de corte em condições naturais de estresse por calor

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ABSTRACT

The objective was to evaluate the supplementation of organic zinc and vitamin E, isolated or in association, in the diet of broiler chickens from 22 to 42 days of age, under natural conditions of heat stress, on the productive performance, carcass, noble cuts, and abdominal fat yield. A group of 720 birds was housed at 22 days of age, distributed in random blocks with a 2x3 factorial design, with two levels of zinc in the organic form (0.0 mg/kg and 120 mg/kg), associated with three levels of vitamin E in the form of DL- α -tocopherol acetate (0.0 mg/kg; 300 mg/kg, and 600 mg/kg), with six replicates, and 20 birds per box. In natural conditions of heat stress, the association of organic zinc and vitamin E in the diet of broilers from 22 to 42 days of age did not affect the productive performance, carcass, noble cuts, and abdominal fat yield. In an isolated way, the supplementation of vitamin E improved the productive performance in from 22 to 33 days of age. Moreover, in the period of 22 to 42 days of age, the level of 312.5 mg/kg of vitamin E provided better creative viability.

RESUMO

Objetivou-se avaliar a suplementação de zinco orgânico e da vitamina E, de forma isolada ou associada, em dietas para frangos de corte no período de 22 a 42 dias de idade, mediante condições naturais de estresse por calor, sobre o desempenho produtivo, rendimentos de carcaça e de cortes nobres, além da gordura abdominal. Alojou-se 720 aves aos 22 dias de idade, distribuídas em delineamento em blocos ao acaso com esquema fatorial 2x3, sendo dois níveis de zinco, na forma orgânica (0,0 e 120 mg/kg) associados a três níveis de vitamina E, na forma de acetato de DL- α -tocoferol (0,0; 300 e 600 mg/kg), seis repetições e 20 aves por boxe. Em condições naturais de estresse por calor, a associação de zinco orgânico e da vitamina E em dietas para frangos de corte no período de 22 a 42 dias de idade, não apresenta efeitos sobre o desempenho produtivo, rendimentos de carcaça e de cortes nobres, além da gordura abdominal. De forma isolada, a suplementação de vitamina E para frangos de corte, melhora o desempenho produtivo na fase de 22 a 33 dias de idade. Ademais, no período de 22 a 42 dias de idade, o nível de 312,5 mg/kg de vitamina E propicia melhor viabilidade criatória.

INTRODUCTION

In regions where high temperatures predominate, the zootechnical farming of several animal species is impaired, especially broilers, which have a deficient thermoregulatory system (MELO et al., 2016). In this

Research with functional nutrients is conducted in order to seek a nutritional strategy that can mitigate the

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harmful effects of heat on birds. Thus, minerals and vitamins become an important source of study, due to their immunomodulatory and antioxidant actions.

Zinc exerts catalytic, structural and regulatory functions in the body of the bird, when it is in deficiency, there is reduction in growth, feed efficiency, hatching rate, skeletal formation, spermatogenesis and feather acquisition (NAZ et al., 2016). Minerals in their isolated form are generally less available, but when organic sources are associated with them, they increase their suitability, providing better absorption, expression of the animal genotype (ABD EL-HACK et al., 2017), and less excretion to the environment (NYS et al., 2018).

Vitamin E stands out for being a liposoluble antioxidant present in large quantities in cell membranes, preventing the action of free radicals on polyunsaturated fatty acids of biological membranes, and can be an important aid in the immune response, performance, and quality of meat (POMPEU; CAVALCANTI; TORAL, 2018).

Some minerals and vitamins synergistically play various organic functions. Zinc and vitamin E have similar functions, being important for immune responses, for their antioxidant characteristics, and possible improvements in productive performance (KAKHKI; BAKHSHALINEJAD; SHAFIEE, 2016).

Thus, the objective was to evaluate the supplementation of organic zinc and vitamin E, isolated or in association, in the diet of broilers from 22 to 42 days of age, in natural conditions of heat stress, on the productive performance, carcass, noble cuts, and abdominal fat yield.

MATERIAL AND METHODS

The research was developed in the Performance Sheds of the Poultry Sector of the Department of Zootechnics of the Center of Agricultural Sciences of the Federal University of Piauí- UFPI, with procedures approved by the Ethics Committee on Animal Experimentation CEEA/UFPI, under the prototocol number 087/12.

In the pre-experimental period (1 to 21 days of age), the birds were kept in conventional built brick sheds, consuming standard feed for the specific stages of rearing, formulated to meet their nutritional requirements, according to Rostagno et al. (2011) and managed in accordance with the rearing standards of the lineage worked (ROSS-AVIAGEN, 2012). The birds were vaccinated in the hatchery against Marek and Gumboro diseases.

On the 22nd day, 720 broilers of the Ross lineage were individually selected, in mixed flocks, being 10 males and 10 females in each box, with an average weight of 1.00 kg distributed in 36 boxes, each one with 2.70 m² each. The experimental design was in random blocks, according to the arrangement of the sheds, in a 2x3 factorial scheme, with two levels of zinc in the organic

form (0.0 mg/kg and 120 mg/kg), associated to three levels of vitamin E, in the DL- α -tocopherol acetate form (0.0 mg/kg; 300 mg/kg and 600 mg/kg), consisting of six treatments and six replicates, with 20 birds per experimental unit.

The boxes were arranged in two brick built sheds, covered with clay tiles, cemented floor, containing plastic curtains on the sides to control temperature and air currents. The bedding used was rice husk, approximately 5 cm thick. Each box contained a tubular feeder and a pendular water dispenser, with feed and water ad libitum. The water was changed twice a day to avoid heating or fermentation of organic material..

The monitoring of the temperature and relative humidity of the air in the warehouses was done by means of a digital thermohygrometer, using dry bulb (Tbs), wet bulb (Tbu) and black bulb (Tgn) thermometers, located at the center of the shed at the height of the back of the birds. Two daily readings (8 and 16 hours) were performed, except for the digital thermometer, in which only one reading was made in the morning, during the whole experimental period. These data were later converted into the Globe Temperature and Humidity Index (ITGU), as proposed by Buffington et al. (1981), in which ITGU = 0.72 (Tbu + Tgn) + 40.6 in $^{\circ}$ C. The light program adopted was the continuous (24 of natural + artificial light), using fluorescent lamps in order to stimulate food consumption by the animals.

The experimental diets (Table 1) were isonutritive and isoenergetic formulated, as recommended by Rostagno et al. (2011). The diets were formulated as follows: T1 - without zinc and vitamin E supplementation; T2 - 0.0 mg/kg of zinc and 300 mg/kg of vitamin E; T3 - 0.0 mg/kg of zinc and 600 mg/kg of vitamin E; T4 - 120 mg/kg of zinc and 0.0 mg/kg of vitamin E; T5 - 120 mg/kg of zinc and 300 mg/kg of vitamin E and T6 - 120 mg/kg of zinc and 600 mg/kg of vitamin E. The quantities of organic zinc and vitamin E were added to those already contained in the mineral and vitamin premix.

Performance parameters were evaluated: feed consumption, weight gain, feed conversion, breeding viability, and production efficiency index in the periods from 22 to 33 and 22 to 42 days of age of the birds, as well as carcass, noble cuts, and abdominal fat yield at 42 days of age.

Feed consumption in both phases was calculated through the difference between the amount of feed supplied at the beginning of the trial period and the leftovers, considering the correction of consumption by mortality, as proposed by Sakomura; Rostagno (2016). To determine the weight gain, the birds were weighed at the beginning and at the end of each phase and the difference between the final average weight and the initial average weight was calculated. The feed conversion of the animals was obtained from the feed consumption data and weight gain.

Table 1. Experimental feed for broilers in the growth phase, 22 to 33 days and final phase, 34 to 42 days of age.

Ingredients	Growth (22-33 days)	Final (34-42 days)		
Corn	63.840	68.238		
Soybean meal 48%	29.560	25.700		
Vegetable Oil	2.820	2.702		
Dicalcium phosphate	1.680	1.055		
Calcitic limestone	0.560	0.740		
NaCl	0.480	0.482		
L-Lysine - HCL (79%)	0.200	0.233		
DL-methionine (99%)	0.160	0.150		
Vitamin-mineral Premix 1,2	0.400	0.400		
Kaolin (inert material)	0.300	0.300		
TOTAL	100	100		
Calculated composition				
Gross Protein (%)	19.500	18.000		
ME (kcal/kg)	3.100	3.150		
Digestible lysine (%)	1.078	1.010		
Digestible methionine (%)	0.431	0.404		
Digestible treonine (%)	0.660	0.608		
Digestible Tryptophan (%)	0.212	0.191		
Calcium (%)	0.732	0.638		
Available phosphorus (%)	0.420	0.298		
Sodium (%)	0.210	0.210		

¹Growth. Guarantee levels per kg of product: Vitamin A: 4.000.000 IU, vitamin D3: 1.100.000 IU, vitamin E: 8.000 IU, vitamin K3: 1.300 mg, vitamin B1: 900 mg, vitamin B2: 3.000 mg, vitamin B6: 1.500 mg, vitamin B12: 6. 000 mcg, niacin: 15 g, pantothenic acid: 5,500 mg, folic acid: 400 mg, biotin: 25 mg, choline: 67.6 g, manganese: 40 g, zinc: 35 g, iron: 25 g, copper: 10 g, iodine: 550 mg, selenium: 125 mg, ethoxyquinol: 26.6 g, monensin: 50 g.

²Final. Guarantee levels per kg of the product: Vitamin A: 2.000.000 IU, vitamin D3: 550.000 IU, vitamin E: 4.500 IU, vitamin K3: 650 mg, vitamin B1: 400 mg, vitamin B2: 1.500 mg, vitamin B6: 750 mg, vitamin B12: 3.500 mcg, niacin: 10 g, pantothenic acid: 3,000 mg, folic acid: 200 mg, biotin: 15 mg, choline: 62.4 g, manganese: 40 g, zinc: 35 g, iron: 25 g, copper: 10g, iodine: 550 mg, selenium: 125 mg, ethoxyquin: 26.6 g.

The viability of the rearing (Vb) and the productive efficiency index (PEI) were calculated with the following formulas: Vb = 100 - (% of dead birds) and PEI = (Weight gain x Vb) / (days until the end of the experiment x Food Conversion) x 100.

At 42 days of age, all birds were weighed and two birds from each experimental unit, with body weight close to the average of the plot (\pm 10%) were submitted to a sixhour fast. After this period, the birds were weighed again to obtain the live weight and later they were slaughtered according to the procedures recommended by the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin - RIISPOA (BRAZIL, 2017), for evaluation of the carcass, noble cuts, and abdominal fat yield.

The carcass yield was determined by the relation between the weight of the eviscerated carcass (without feet, head, and neck) and the live weight of the birds on the slaughter platform. In addition, the yield of the noble cuts (breast, drumstick, and thigh) and abdominal fat (fat tissue around the Bursa of Fabricius, proventriculus, gizzard, and cloaca) were determined, being weighed and their yield calculated compared to the weight of the eviscerated carcass.

The average of environmental variables were calculated. The parameters of productive performance, characteristics of the carcass and noble cuts, and of the abdominal fat were submitted to the evaluation of homogeneity and normality, with the identified outliers being removed. Later, the data were submitted to Analysis of Variance, and when significant, the levels of organic zinc were compared by the Tukey test and for vitamin E levels, polynomial regression was used according to the statistical procedures of the SAS software (2002), with a significant $\alpha = 0.05$.

RESULTS AND DISCUSSION

The average values of temperature, relative humidity, and ITGU recorded during the 22 to 33 day-old phase of the birds varied from 30.0 ± 0.76 °C; $57.7\pm1.88\%$, and 80.1 ± 1.34 , respectively, and in the phase from 34 to 42 days of age ranged from 30.7 ± 0.49 °C; $58.9\pm3.77\%$, and 81.1 ± 1.15 , respectively.

The animals passed the thermoneutral zone, being exposed to the natural conditions of heat stress. The comfort temperature indicated for birds from the 3rd week of life is 21 to 25 °C (PAULINO et al., 2019), the relative air humidity should be between 60 to 70% (SOUZA et al., 2018), and the ITGU ranging from 65 to 77 (STAUB et al., 2016).

When birds are under heat stress, there is an increase in energy demand to ensure the maintenance of homeothermia. In addition, when stress is lasting, there is an increase in glycogenesis, production of ketone bodies, gastric lesions, suppression of appetite, and impairment of immunological mechanisms, resulting in a drop in productivity (SANTOS et al. 2017).

High air temperature values associated with levels of relative humidity below the comfort range result in airway desiccation, as well as in the dehydration of the birds. These physical factors directly influence feed consumption, weight gain, and feed conversion (QUEIROZ et al., 2017).

In the growth phase of birds (22 to 33 days of age), there was no interaction (P>0.05) between the factors, zinc, and vitamin E levels for the performance variables (Table 2).

Parameters	Zinc Levels	Levels	Levels of Vitamin E (mg/kg)			CV^1	P ² value	
	(mg/kg)	0	300	600	Mean	(%)	L	Q
	0	1.72	1.72	1.70	1.71	2.39		
Feed intake (kg/poultry)	120	1.69	1.72	1.69	1.70			
(kg/poundy)	Mean	1.71	1.72	1.69			0.451	0.123
Weight gain (kg/poultry) —	0	0.96	0.97	0.98	0.97	3.93		
	120	0.95	0.99	1.01	0.98			
	Mean	0.96	0.98	0.99			0.032	0.763
	0	1.79	1.77	1.74	1.77	3.31		
Food conversion	120	1.78	1.74	1.68	1.73			
-	Mean	1.78	1.76	1.71			0.003	0.471
Viability of rearing (%) –	0	98.33	99.17	99.17	98.89	3.03		
	120	97.50	99.17	96.67	97.78			
	Mean	97.92	99.17	97.92			1.000	0.245
Productive Efficiency Index –	0	443.17	454.13	467.30	454.86	7.22		
	120	438.17	469.94	486.97	465.03			
	Mean	440.67	462.04	477.1			0.012	0.792

π_{-1}		fed with different levels of zinc and vitamin E.
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Tuble 2. differen perior manee	, nom 22 to 55 days of age,	

¹ CV = coefficient of variation.

² L, Q: probability of linear and quadratic order relative to vitamin E levels.

In isolation, the addition of organic zinc did not influence (P>0.05) the feed consumption, weight gain, feed conversion, viability of the rearing, and the production efficiency index of birds (Table 2).

The role of zinc in poultry nutrition is important for several metabolic pathways, being present in most of the organic tissues. Its absorption occurs in the enterocyte, where it binds to metallothionine, a hepatic protein that has a high affinity for zinc and regulates the amount of zinc entering the organism. In the plasma, about two thirds of the zinc binds to albumin and is readily used by the tissues. At the cellular level, Zip-type transporters perform zinc absorption and its excretion involves ZnT transporters (GOFF, 2018). The results indicated that the level of zinc contained in the mineral premixes used in the formulations met the requirements for broiler chickens to perform these relevant functions. In this case, additional doses of organic zinc did not exert positive effects on the performance of the animals that were under heat stress, since birds raised in high temperature environments reduce feed intake in an attempt to decrease metabolic heat production (LOPES et al., 2015). Results contrary to this research were obtained by Boiago et al. (2013), who reported that the use of microminerals complexed to organic molecules, such as zinc, provided a better productive performance, especially when the birds were bred in a hot environment.

The amount of vitamin E presented effects (P<0.05) on weight gain, feed conversion, and in the Productive Efficiency Index of broiler chickens (Table 2). Regarding weight gain, a linear increase was observed (P<0.05) according to the equation ($\hat{y} = 0.9622 + 0.00006x, R^2 = 0.98$), in which there was an increase in weight gain as the vitamin E levels in the diet increased. There was a linear decreasing effect (P<0.05) for food conversion, according to the equation ($\hat{y} = 1.7877 - 0.0001x, R^2 = 0.95$).

The greatest weight gain can be related to the benefits of vitamin E supplementation in broilers under heat stress due to its antioxidant properties and also because of the reduction of plasma concentrations of corticosterone that cause protein degradation in the skeletal muscle, affecting broiler performance (YOO et al., 2016). Zeferino et al. (2016) noted that the antioxidant action of vitamin E provided a beneficial effect in broilers at 28 and 42 days of age when reared in high temperature environments (32°C).

The benefits of vitamin E on the feed conversion of birds were observed in several researches (SELVAM et al., 2017; ZEFERINO et al., 2016), using different levels of supplementation, from 70 mg/kg to 93 mg/kg.

A linear increase was observed (P<0.05), according to the equation $\hat{y} = 440,63 + 0,0813x$ (R² = 0.97) in which there was an increase in the Productive Efficiency Index as the levels of vitamin E in the diets increased. This index is totally related to the productive variables, in this case, the increase in the weight gain and the improvement in the feed conversion of the birds supplemented with vitamin E, also reflected in the increase of the Productive Efficiency Index. Thus, vitamin E supplementation alleviated the deleterious effects triggered by heat stress in the broilers, since these animals may present a reduced antioxidant pattern (SILVA et al. 2015). This explains the beneficial action of this vitamin in broiler chickens, being an essential nutritional element for the health and growth of these animals. In addition, the PEI is also used as a basis for the income of producers, and the higher its rate the higher the payment to the producer and the higher the profit of the company. Opposite results were obtained by Albuquerque et al. (2017) when analyzing diets containing 0.1 mg/kg and 0.3 mg/kg of organic selenium associated with different levels of vitamin E (300 mg/kg, 400 mg/kg, and 500 mg/kg) for poultry at 22 to 33 days of age, who did not observe improvement in the parameters of feed consumption, weight gain, feed conversion, and Production Efficiency Index.

In the termination phase of the poultry (22 to 42 days of age) there was no interaction (P>0.05) between the factors, zinc, and vitamin E levels, for the performance variables (Table 3).

Table 3. Performance	of broiler chickens.	from 22 to 42 day	s of age, fed with	different levels of zinc and	d vitamin E.

Parameters	Zinc	Vitamin E Levels (mg/kg)			Mean	CV^1	P ² values	
	Levels - (mg/kg)	0	300	600		(%)	L	Q
Feed intake	0	3.04	3.10	3.03	3.06	4.77		
(kg/poultry)	120	2.98	3.15	3.04	3.06			
	Means	3.01	3.13	3.03			0.715	0.047
Weight gain (kg/poultry)	0	1.77	1.66	1.61	1.68	7.88		
	120	1.60	1.69	1.66	1.65			
	Means	1.69	1.67	1.63			0.334	0.750
Food conversion	0	1.72	1.87	1.88	1.83	6.18		
	120	1.86	1.87	1.83	1.85			
	Means	1.79	1.87	1.86			0.227	0.344
Viability of rearing	0	90.0	94.17	93.33	92.50	4.59		
	120	89.1	93.33	89.17	90.56			
	Means	89.5	93.75	91.25			0.339	0.032
Productive Efficiency Index	0	448.67	399.87	382.09	410.21	15.68		
	120	368.75	404.06	386.82	386.55			
	Means	408.71	401.97	384.46			0.349	0.809

¹ CV = coefficient of variation.

² L, Q: probability of linear and quadratic order relative to vitamin E levels.

In isolation, the inclusion of organic zinc did not affect (P>0.05) feed consumption, weight gain, feed conversion, breeding viability, and the Production Efficiency Index of poultry (Table 3). Corroborating the present study, Zakaria et al. (2017) found no significant effects for any of the zinc sources (80 mg/ kg of zinc oxide and 122 mg/kg of Availa-Zn) evaluated in the performance of broilers.

Vitamin E levels resulted in quadratic effects (P<0.05) on feed consumption and on the viability of rearing of the broiler chickens (Table 3), according to the respective equations: $\hat{y} = 3,016 + 0,0008x - 0,000001x^2$, R² = 0.92 and $\hat{y} = 404,69 + 9,4025x - 5,3825x^2$, R² = 0.93.

The highest feed intake was obtained with 400 mg/kg of vitamin E, although it is important to note that a high

amount of this vitamin can be toxic to cells, therefore causing a drop in viability by increasing its inclusion in the diet. The best viability of rearing was obtained with 312.5 mg/kg. This parameter is related to the decrease of mortality of the broilers, and this in turn may be linked to their health and immunity improvement, since vitamin E is critical to the primary immune response, interacting with the components of the immune system, and its deficiency affects negatively the immunity of animals.

At 42 days of age there was no interaction (P>0.05) between the factors, zinc, and vitamin E levels for carcass, noble cuts, and abdominal fat yield (Table 4).

Table 4. Carcass and noble cuts yield and percentage of abdominal fat from broilers fed with different levels of zinc and vitamin E.

Parameters	Zinc Levels	Vitamin E Levels (mg/kg)			Means	CV^1	P ² values	
	(mg/kg)	0	300	600	_	(%)	L	Q
	0	81.85	83.07	82.41	82.44	3.53		
Carcass yield (%)	120	80.55	82.21	82.54	81.77			
	Means	81.20	82.64	82.47			0.133	0.272
	0	32.01	30.38	31.27	31.22	4.86		
Chest yield (%)	120	31.91	31.03	30.29	31.08			
	Means	31.96	30.70	30.78			0.067	0.222
	0	12.90	13.12	12.93	12.99	4.44		
Drumstick yield (%)	120	13.32	12.77	13.09	13.06			
	Means	13.11	12.94	13.01			0.685	0.570
Thigh yield (%)	0	12.94	12.37	12.38	12.56	10.26		
	120	12.64	11.98	12.03	12.21			
	Means	12.79	12.17	12.20			0.265	0.475
Abdominal fat (%)	0	2.28	1.92	2.07	2.09	22.74		
	120	2.17	2.15	1.98	2.10			
	Means	2.23	2.03	2.02			0.306	0.597

 1 CV = coefficient of variation.

² L, Q: probability of linear and quadratic order relative to vitamin E levels.

The inclusion of organic zinc alone did not influence (P>0.05) carcass, noble cuts, or the abdominal fat of birds (Table 4). Similarly, a study conducted by Zakaria et al. (2017), revealed that the bird carcass yield was not significantly influenced by treatments (80 mg of inorganic zinc and 80 mg of zinc with 42 mg of an organic amino acid complex).

In isolation, vitamin E levels did not influence (P>0.05) the carcass, noble cuts, or the abdominal fat of broilers (Table 4). Barbosa Filho et al. (2017) concluded that the use of canola oil alone or combined with vitamin E did not affect the productive characteristics of the carcass, cuts, or the humoral immune response in broilers compared to soybean oil. In contrast, Zeferino et al. (2016), employing diets containing vitamin E (93 mg/kg to 109 mg/kg) and vitamin C (257 mg/kg to 288 mg/kg), observed that the exposure of chickens to a heat stress (32° C) between the 28th and 42nd days of rearing increased the carcass and abdominal fat percentages.

In regions with high temperatures, the animals tend to decrease feed consumption and there may be less muscle formation, resulting in more energy availability for abdominal fat deposition, affecting the parameters of carcasses (LOPES et al., 2015).

CONCLUSIONS

In natural conditions of heat stress, the association of organic zinc and vitamin E in diets for broilers from 22 to 42 days of age, had no effect on productive performance; carcass and noble cuts yield, besides abdominal fat. In an isolated way, vitamin E supplementation improved the productive performance in the phase of 22 to 33 days of age. Moreover, in the period of 22 to 42 days of age, the level of 312.5 mg/kg of vitamin E provides better viability of rearing.

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