Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2636
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v38i4.31498

Black pepper (*Piper nigrum*) in diets for laying hens on performance, egg quality and blood biochemical parameters

Ramon Duque Melo, Frank George Guimarães Cruz*, Julmar da Costa Feijó, João Paulo Ferreira Rufino, Lucas Duque Melo and Jessica Lima Damasceno

*Departamento de Produção Animal e Vegetal, Setor de Avicultura, Faculdade de Ciências Agrárias, Universidade Federal do Amazonas, Av. Gal. Rodrigo Otávio Jordão Ramos, 3000, 69077-000, Manaus, Amazonas, Brazil. *Autor para correspondência: frankgcruz@gmail.com*

ABSTRACT. The objective of this study was to evaluate the increasing levels (0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%) of black pepper in diets for laying hens on performance, egg quality and blood biochemical parameters. Hissex White hens (n=168) at 30 weeks of age were used. The experimental method was completely randomized with seven treatments with four replicates of six birds each. Estimates of black pepper levels were determined by polynomial regression. The performance showed no significant differences ($p > 0.05$). The eggshell percentage was significantly influenced ($p < 0.05$), in which the level of 0.30% inclusion impaired eggshell quality. Triglycerides level increased significantly ($p < 0.05$), according to increasing levels of black pepper in the diet. It can be concluded that black pepper can be used in diets for laying hens as phytogenic additive without harming the performance. However, this inclusion causes a reduction in eggshell percentage and an increase in the level of triglycerides in the bloodstream.

Keywords: alternative food, eggshell, phytogenic, piperine, serum metabolism.

Pimenta do reino (*Piper nigrum*) na dieta de poedeiras leves sobre o desempenho, qualidade do ovo e análise bioquímica sanguínea

RESUMO. Objetivou-se com este trabalho avaliar a inclusão de níveis crescentes (0; 0,1; 0,2; 0,3; 0,4; 0,5 e 0,6%) de pimenta do reino na dieta de poedeiras comercias leves sobre o desempenho, a qualidade do ovo e os parâmetros bioquímicos sanguíneos. Foram utilizadas 168 poedeiras da linhagem Hissex White com 30 semanas de idade. O delineamento experimental foi o inteiramente casualizado com sete tratamentos contendo quatro repetições de seis aves cada. As estimativas dos níveis de pimenta do reino foram determinadas por meio de regressão polinomial. As variáveis de desempenho produtivo não apresentaram diferenças significativas ($p > 0,05$). A percentagem de casca foi influenciada significativamente ($p < 0,05$) onde o nível de 0,30% de inclusão de pimenta do reino piorou a qualidade da casca dos ovos. A concentração de triglicerídeos apresentou diferenças significativas ($p < 0,05$) onde a inclusão de pimenta do reino ocasionou aumento crescente da concentração de triglicerídeos no sangue das aves. Concluiu-se que a pimenta do reino em dietas para poedeiras leves pode ser utilizada como aditivo fitogênico sem ocasionar alterações no desempenho produtivo. Todavia, com o advento desta inclusão, a pimenta do reino apresenta redução do percentual de casca do ovo e incremento na concentração de triglicerídeos na corrente sanguínea.

Palavras-chave: alimento alternativo, casca do ovo, fitogênico, metabolismo sérico, piperina.

Introduction

The concern about animal production is increasingly directed to the quantity and quality of the final product, and its implications for food security, environment and animal welfare. The importance of the interaction between nutrition and health is essential in an animal production system, although factors related to the environment and management also influences the productive performance of the individual and the herd (Gonçalves et al., 2010). In this context, the additives are used in animal nutrition aiming at animal welfare and maximum performance, and are not harmful to animals and humans, should not leave residues in consumer products and should not contaminate the environment (Russell & Houlihan, 2003). Among the most currently studied and used additives stand out organic acids (Mroz, 2005), plants and their extracts (Santurio et al., 2007; Valero et al., 2016; Valero et al., 2014), enzymes (Martins, Vieira, Berchielli, Prado, & Paula, 2007; Martins, Vieira, Berchielli, Prado, & Garcia, 2006), probiotics and prebiotics (Maiorka, Santin, Sugeta, Almeida, & Macari, 2001), which hitherto have shown promising results (Santurio et al., 2007).

Phytogenic additives have gained the attention of researchers, because they act preventing common diseases in animals and also in maintaining health. Phytogenics are also of interest to consumers because they are considered natural alternatives to synthetic compounds (Pearce & Jin, 2010).

Known worldwide, black pepper is commonly used as a seasoning or ingredient in alternative medicine, because of its active ingredient called piperine. This compound has potential application as a natural additive directed to animal production, as it has several advantages, among which highlights the fact that it is a natural product that can be found in large quantities at low production cost (Parmar et al., 1997; Tatli, Seven, Yılmaz, & Şimşek, 2008). In addition, it presents a potential metabolization which, in mammals, leaves no residue in the animal organism, different from that recommended with the use of antibiotics, having antioxidative, anti-apoptotic and cell recovery activities, suggesting therapeutic use in conditions of compromised immune system (Cardoso et al., 2009).

Cardoso et al. (2009) also stated that the administration of piperine directly in the diet for broilers did not cause mortality in the flock or clinical changes in the general condition of the animals. The results suggest that administration of 1.12 mg piperine per kg body weight for 14 days shows no toxic effects for broilers and stimulates the number of heterophile. At higher doses, there are histopathological changes in the tissues analyzed, and a significant increase of total and specific number of leukocytes.

Considering the above, the present study aimed to evaluate the increasing levels of black pepper in diets for light commercial laying hens on performance, egg quality and blood biochemical parameters.

Material and methods

The experiment was conducted in the facilities of Poultry Sector, Department of Animal and Plant Production (DPAV), Faculty of Agrarian Sciences (FCA), Federal University of Amazonas (UFAM), located in the south sector of the university campus, Manaus, State of Amazonas, Brazil.

The experimental period consisted of 84 days divided into four periods of 21 days each. Before the onset of the experiment, broilers were adapted to feed and facilities for seven days.

The experimental poultry house had 17.0 m in length and 3.5 m in width, containing galvanized wire cages, trough feeders and nipple drinkers. Hissex White laying hens (n = 168) at 33 weeks of age were weighed at the beginning of the experiment in order to standardize the experimental plots, presenting an average weight of 1.54 ± 0.16 kg. The egg collection was performed twice a day (9 and 15 hours), with record of each occurrence (mortality, number of eggs etc).

Hens were arranged in a completely randomized design consisting of seven treatments corresponding to levels of inclusion of black pepper (0; 0.1; 0.2; 0.3; 0.4; 0.5 and 0.6%), with four replicates of six birds per treatment. Throughout the experimental period, birds were exposed to 16 hours of light day^{-1} (12 hours of natural light + 4 hours of artificial light).

Fresh, whole black pepper was ground to be supplied to birds. Isonutritive diets were formulated using the software Supercrac so as to meet the nutritional requirements of the birds and according to values of the ingredients provided by the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011), with the exception of composition of black pepper and are listed in Table 1.

In animal performance, for each period, were evaluated feed intake (g bird^{-1} day^{-1}), egg production (%), egg mass (g), feed conversion (kg feed per kg^{-1} produced egg^{-1}) and feed conversion (kg^{-1} feed dozen eggs^{-1}). In the last two days of each period, were collected at random four eggs each in plot to determine egg quality, in which were evaluated the egg weight (g), albumen weight (%), yolk weight (%), albumen height (mm), yolk height (mm), shell weight (%), shell thickness (μm), specific gravity (g cm^{-3}) and yolk color. Before evaluation, eggs were stored for one hour to equalize the temperature to room temperature.

Eggs were weighed on an electronic scale accurate to 0.01 g. The egg mass was obtained by calculating the ratio between egg weight and egg production multiplied by a hundred. Whole eggs after weighing were placed in wire baskets and immersed in plastic buckets with different levels of sodium chloride (NaCl), from the lowest to the highest concentration with density variations from 1.075 to 1.100 g cm^{-3}, at 0.005 interval. The eggs were taken when floated to the surface, and had the respective values recorded.

For the analysis of albumen and yolk weight, were used a manual separator of albumen and yolk. The albumen and yolk were placed in plastic cups, both tare weighed in an analytical balance, and weighed. To calculate the height of albumen and yolk, they were placed on a flat glass plate to determine their respective values. The procedure for albumen and yolk height measurement is to measure in the median area, between the outer edge of the albumen and yolk. To measure the heights,

Table 1. Composition of diets containing black pepper.

	Levels of black pepper (%)						
Ingredients	0	0.1	0.2	0.3	0.4	0.5	0.6
Corn (7.88%)	62.303	62.187	62.071	61.955	61.839	61.722	61.606
Soybean Meal (46%)	25.657	25.674	25.691	25.708	25.725	25.742	25.759
Limestone	9.240	9.239	9.237	9.236	9.235	9.233	9.232
Dicalcium phosphate	1.695	1.695	1.695	1.695	1.695	1.695	1.695
Premix[1]	0.500	0.500	0.500	0.500	0.500	0.500	0.500
DL-methionine (99%)	0.255	0.255	0.255	0.255	0.255	0.255	0.255
Black pepper	0.000	0.100	0.200	0.300	0.400	0.500	0.600
Salt	0.350	0.350	0.350	0.350	0.350	0.350	0.350
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient	Nutritional levels						
E.M, kcal kg^{-1}	2,697	2,696	2,694	2,692	2,693	2,690	2,689
Crude protein, %	17.000	17.000	17.000	17.000	17.000	17.000	17.000
Methionine, %	0.520	0.520	0.520	0.520	0.520	0.520	0.520
Methionine+ cystine, %	0.786	0.786	0.786	0.786	0.786	0.786	0.786
Lysine, %	0.861	0.862	0.862	0.862	0.862	0.862	0.863
Threonine, %	0.659	0.659	0.659	0.659	0.659	0.659	0.659
Tryptophan, %	0.205	0.205	0.205	0.205	0.205	0.205	0.205
Calcium, %	4.000	4.000	4.000	4.000	4.000	4.000	4.000
Available phosphorus, %	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Sodium, %	0.156	0.155	0.156	0.156	0.156	0.156	0.156

[1]Guarantee levels per kilogram of product: Vitamin A 2,000,000 IU, Vitamin D3 400,000 IU, Vitamin E 2,400 mg, Vitamin K3 400 mg, Vitamin B1 100 mg, Vitamin B2 760 mg, Vitamin B6 100 mg, Vitamin B12 2,400 mcg, Niacin 5,000 mg, Calcium pantothenate 2,000 mg, Folic Acid 50 mg, Coccidiostat 12,000 mg, Choline 50,000 mg, Copper 1,200 mg, Iron 6,000 mg, Manganese 14,000 mg, Zinc 10,000 mg, Iodine 100 mg. Selenium 40 mg. Vehicle Q.S.P. 1,000 g.

were used an electronic caliper, with values expressed in millimeters. The weight of the egg shell was obtained after the same are washed, dried at room temperature for 48 hours and then weighed, in grams.

Shell thickness was determined with dried shell and the reading was made with the aid of a micrometer. Data were collected in three regions of the shell: basal, meridional and apical, and the values were recorded. From the values obtained in the three regions, were calculated the average thickness of the egg shell in micrometer. To evaluate the color of egg yolk, were used the Roche color fan, ranging from 1 to 15. The determination of the Haugh unit used the methodology proposed by Nogueira, Cruz, Tanaka, Rufino and Santana (2014).

In the analysis of biochemical parameters, two birds of each treatment were randomly selected, and subjected to blood collection, approximately 3 mL from the ulnar vein, located on the wing of each bird, according to the methodology proposed by Bezerra et al. (2015). The collected blood was immediately sent to the Poultry Technology Laboratory, Poultry Sector, UFAM, to determine the levels of glucose, triglycerides, cholesterol and pH using a portable biochemical analyzer (Accucheck Trend, ROCHE) with the aid of reagent strips specific to each analysis, except for the pH, which was determined with a pH meter (SENTRON, 1001) coupled to a probe (SENTRON LanceFET, 1074-001) with fine tip penetration inserted directly in blood samples.

Statistical analysis was performed using the software Statistical Analysis System - SAS (2004) and estimates of the treatments were subjected to polynomial regression at 5%.

Results and discussion

There were no significant differences ($p > 0.05$) between the means of production performance variables (Table 2) with the inclusion of black pepper in the feed. These results diverge from those observed by Pinheiro et al. (2012), who included forage radish as a feed additive for laying hens, and observed decrease in productive performance. The authors also claim that when used alternative foods, including phytogenic compounds with active biomolecules, it should be taken into account increases or decreases in production performance, which is essential when considering the inclusion of these in poultry diets.

According to Jamroz, Wertelecki, Houszka and Kamel (2006), phytogenic additives in feeding broiler can help the functions of the intestinal tract due to stimulation of mucus secretion in the intestine, which impairs the adhesion of pathogenic microorganisms and allows maintenance of normal microbiota of the intestinal tract. In laying hens, the results are even scarcer, but, it was observed that the inclusion of up to 0.60% does not directly influence the performance, which according to Galal, El-Motaal, Ahmed and Zaki (2008), working with the addition of garlic in diets for laying hens, even if not affecting the production performance of poultry, the inclusion of phytogenic additives in feeding of these birds can promote a number of positive side effects in production, such as improvement in egg quality, and in physiological parameters, among others.

Table 2. Feed intake (CR), laying percentage (PERCP), feed conversion kg of feed per kg of produced egg (CA, kg kg^{-1}), feed conversion kg of feed per dozen eggs produced (CA, kg dz^{-1}) and egg mass (MO) of laying hens fed diets with increasing levels of black pepper.

Variables	Levels of inclusion of black pepper (%)							p-value	Effect	CV, %
	0.00	0.10	0.20	0.30	0.40	0.50	0.60			
CR, g $bird^{-1}$ day^{-1}	101.49	104.02	101.95	100.17	102.55	98.01	99.09	0.88	ns	6.69
PERCP, %	95.98	96.47	95.28	96.13	90.72	93.94	94.94	0.15	ns	3.14
CA, kg kg^{-1}	2.36	2.41	2.32	2.34	2.51	2.32	2.32	0.82	ns	8.87
CA, kg dz^{-1}	1.28	1.29	1.28	1.25	1.36	1.25	1.25	0.76	ns	8.39
MO, g	54.08	54.27	55.33	53.86	51.68	53.34	53.70	0.47	ns	4.21

CV – Coefficient of variation. p-Value – Coefficient of Probability. ns – non-significant.

Toghyani, Toghyani, Gheisari, Ghalamkari and Mohammadrezaei (2010) investigated the performance of broilers fed black pepper and mint and observed an increase in weight gain at 28 days of age, when the broilers were supplemented with 4 g kg^{-1} mint, although the final weight at 42 days of age has not changed.

Significant differences ($p < 0.05$) were detected (Table 3) in shell percentage between the treatments. There was a quadratic effect ($Y = 0.251x^2 + 0.143x + 10.04$ $R^2 = 0.64$), where from the derivation of the function, were observed the point of best shell percentage (10.04%) at 0.28% level of black pepper inclusion in the diets. These results were superior to those obtained by Fernandes, Murakami, Scapinello, Moreira, and Varela (2009), when evaluated the inclusion of different levels of vitamin K supplementation in the diet for HyLine® W36 laying hens at 67 weeks of age.

According to Parmar et al. (1997) and Toghyani et al. (2010), piperine has biomolecular functions similar to those given by various compounds, including vitamin K. Once the use of phytogenic substances presenting active biomolecular compounds with similar functions to synthetic compounds, in addition to reduce the use of these, they can also aid in the regulation of various metabolic functions and therefore increase production of poultry.

For Trindade, Nascimento and Furtado (2007), the integrity of the shell has great influence on egg quality, and is one of the factors that producers are more concerned about, because the shell quality may vary due to many factors, and among these, the use of phytogenic additives, such as black pepper, may mean increases in egg quality without the need to use synthetic compounds in the feed.

In the other variables of egg quality, no significant differences were found ($p > 0.05$), in which the results of these were lower to those found by Ting, Yeh, and Lien (2011), who assessed the antioxidant function of adding flavonoids from citrus fruits, such as phytogenic substances at different concentrations, to the diet of laying hens at 22 weeks of age, and also verified no significant differences, indicating that the inclusion of phytogenic additives at different concentrations do not necessarily causes losses in egg quality.

There were significant differences ($p < 0.05$) (Table 4) between values of triglycerides with the addition of different levels of black pepper in diets. There was a quadratic effect ($Y = -48.56x^2 + 76.51x + 228.97$ $R^2 = 0.76$), where from the derivation of the function were observed the point of best triglyceride level (259.08 mg dL^{-1}) at 0.78% level of black pepper inclusion in the diets.

Borsa, Kohayagawa, Boretti, Saito, and Kuibida (2006) argued that with the advent of faster metabolism, light laying hens require greater mobilization of fatty acids and steroid hormones for yolk synthesis than heavier hens, causing significant changes in the level of total triglycerides. Parmar et al. (1997) also claimed that, because piperine is an active ingredient, inclusion of black pepper in diets for poultry can cause metabolic changes, especially in the catabolism of fatty acids and regulation of energy metabolism, which direct affect the physiological response thereof, where the addition of a new element in the diet can cause an increased mobilization of triglycerides from tissues to the bloodstream.

Table 3. Egg weight (PEO), percentage of albumen (PEA), percentage of yolk (PEG), percentage of shell (PEC), albumen height (AA), yolk height (AG), shell thickness (EC), specific gravity (GE), Haugh unit (HU) and yolk color (PG) of eggs from hens fed diets with increasing levels of black pepper.

Variables	Levels of inclusion of black pepper (%)							p-value	Effect	CV, %
	0.00	0.10	0.20	0.30	0.40	0.50	0.60			
PEO, g	56.35	56.24	58.05	56.03	56.96	56.76	56.55	0.41	ns	2.30
PEA, %	57.10	59.25	58.55	58.78	58.39	60.29	60.69	0.11	ns	2.87
PEG, %	27.65	28.47	28.27	28.50	28.27	28.39	28.20	0.06	ns	4.10
PEC, %	10.22	10.14	10.13	10.27	10.18	10.01	9.76	0.02	Q	1.88
AA, mm	5.47	5.71	5.46	5.46	5.81	5.75	5.62	0.07	ns	3.45
AG, mm	15.56	15.62	15.79	15.46	15.65	15.50	15.25	0.27	ns	1.89
EC, μm	0.41	0.40	0.40	0.40	0.40	0.40	0.39	0.18	ns	1.92
GE, g mL^{-1}	1087.42	1086.64	1086.17	1086.48	1086.56	1085.39	1085.85	0.06	ns	0.13
UH	60.73	60.57	62.42	60.42	61.27	61.09	60.90	0.41	ns	2.15
PG	5.43	5.60	5.75	5.54	5.73	5.85	5.78	0.09	ns	3.56

CV – Coefficient of variation. p-Value – Coefficient of Probability. Q – Quadratic Effect ($p < 0.05$). ns – non-significant.

Table 4. Glucose (GLU), triglycerides (TRIG), cholesterol (COL) and blood pH of laying hens fed diets with increasing levels of black pepper.

Variables	Levels of inclusion of black pepper (%)							p-value	Effect	CV, %
	0.00	0.10	0.20	0.30	0.40	0.50	0.60			
GLU, mg dL^{-1}	225.50	218.50	210.50	199.50	232.00	207.50	199.00	0.17	ns	5.81
TRIG, mg dL^{-1}	252.00	408.50	490.00	401.50	474.00	472.50	566.00	0.03	Q	14.73
COL, mg dL^{-1}	163.00	163.00	168.00	165.00	166.50	168.00	167.50	0.57	ns	2.05
pH	6.48	6.52	6.80	6.58	6.54	7.12	6.92	0.29	ns	4.15

CV – Coefficient of variation. p-Value – Coefficient of Probability. Q – Quadratic Effect ($p < 0.05$). ns – non-significant.

Conclusion

In summary, black pepper can be added to diets for laying hens as a phytogenic additive without harming the production performance and egg quality. However, this inclusion causes a reduction in eggshell percentage and an increase in the level of triglycerides in the bloodstream.

References

Bezerra, N. S., Cruz, F. G. G., Costa, A. P. G. C., Rufino, J. P. F., Melo, R. D., Feijó, J. C., Hollerverger, S. V. S. (2015). Óleo de copaíba (*Copaifera* sp.) na alimentação de galos reprodutores semipesados. *Revista Científica de Avicultura e Suinocultura, 1*(1), 1-13.

Borsa, A., Kohayagawa, A., Boretti, L. P., Saito, M. E., & Kuibida, K. (2006). Serum levels of hepatic enzyme function in clinically healthy broiler chickens. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, 58*(4), 675-677.

Cardoso, V. S., Lima, C. A. R., Lima, M. E. F., Dorneles, L. E. G., Teixeira Filho, W. L., Lisboa, S. R., ... Danelli, M. G. M. (2009). Administração oral de piperina em frangos de corte. *Ciencia Rural, 39*(5), 1521-1526.

Fernandes, J. I. M., Murakami, A. E., Scapinello, C., Moreira, I., & Varela, E. V. (2009). Effect of vitamin K on bone integrity and egg shell quality of white hen at the final phase of the laying cycle. *Revista Brasileira de Zootecnia, 38*(3), 488-492.

Galal, A., El-Motaal, A. M., Ahmed, A. M. H., & Zaki, T. G. (2008). Productive performance and immune response of laying hens as affected by dietary propolis supplementation. *International Journal of Poultry Science,* 7(3), 272-278.

Gonçalves, F. M., Corrêa, M. N., Anciuti, M. A., Gentilini, F. P., Zanusso, J. T., & Rutz, F. (2010). Nutrigenômica: situação e perspectivas na alimentação animal. *Revista Portuguesa de Ciencias Veterinarias, 104*, 569-572.

Jamroz, D., Wertelecki, T., Houszka, M., & Kamel, C. (2006). Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *Journal of Animal Physiology and Animal Nutrition, 90*(5-6), 255-268.

Maiorka, A., Santin, E., Sugeta, S. M., Almeida, J. G., & Macari, M. (2001). Utilização de prebióticos, probióticos ou simbióticos em dietas para frangos. *Revista Brasileira de Ciência Avícola, 3*(1), 75-82.

Martins, A. S., Vieira, P. F., Berchielli, T. T., Prado, I. N., & Paula, M. C. (2007). Degradabilidade *in situ* e observações microscópicas de volumosos em bovinos suplementados com enzimas fibrolíticas exógenas. *Revista Brasileira de Zootecnia, 36*(6), 1927-1936.

Martins, A. S., Vieira, P. V., Berchielli, T. T., Prado, I. N., & Garcia, J. A. S. (2006). Eficiência de síntese microbiana e atividade enzimática em bovinos submetidos à suplementação com enzimas fibrolíticas. *Revista Brasileira de Zootecnia, 35*(3), 1194-1200.

Mroz, Z. (2005). Organic acids as potential alternatives to antibiotic growth promoters for pigs. *Advances in Pork Production, 16*, 169-182.

Nogueira, M. A., Cruz, F. G. G., Tanaka, E. S., Rufino, J. P. F., & Santana, T. M. (2014). Óleo de dendê (Elaeais guineensis Jaquim) na alimentação de poedeiras leves. *Revista Acadêmica: Ciências Agrárias e Ambientais, 12*(2), 103-111.

Parmar, V. S., Jain, S. C., Bisht, K. S., Jain, R., Taneja, P., Jha, A., ... Boll, P. M. (1997). Phytochemistry of the genus Piper. *Phytochemistry, 46*(4), 597-673.

Pearce, M., Jin, G. L. Z. (2010). Aditivos Fitogênicos. *Porkworld, 58*, 128-136.

Pinheiro, J. W., Fonseca, N. A. N., Bridi, A. M., Silva, C. A., Oba, A., & Medeiros, L. G. (2012). Desempenho e qualidade dos ovos de poedeiras comerciais leves submetidas a dietas contendo torta de nabo forrageiro (*Raphanus sativus*). *Semina: Ciências Agrárias, 33*(4), 1555-1564.

Rostagno, H. S., Albino, L. F. T., Donzele, J. L., Gomes, P. C., Oliveira, R., & Lopes, D. C. (2011). *Composição de alimentos e exigências nutricionais* (3a ed., Vol. 1). Viçosa: Universidade Federal de Viçosa.

Russell, J. B., & Houlihan, A. J. (2003). Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS Microbiology Reviews, 27*(1), 65-74.

Santurio, J. M., Santurio, D. F., Pozzatti, P., Moraes, C., Franchin, P. R., & Alves, S. H. (2007). Atividade antimicrobiana dos óleos essenciais de orégano, tomilho e canela frente a sorovares de *Salmonella enterica* de origem avícola. *Ciencia Rural, 37*(3), 803-808.

Statistical Analysis System [SAS]. (2004). *SAS/STAT User guide, Version 9.1.2*. Cary, NC: SAS Institute Inc.

Tatli, S. P., Seven, I., Yılmaz, M., & Şimşek, Ü. G. (2008). The effects of Turkish propolis on growth and carcass characteristics in broilers under heat stress. *Animal Feed Science and Technology, 146*(1-2), 137-148.

Ting, S., Yeh, H. S., & Lien, T. F. (2011). Effects of supplemental levels of hesperetin and naringenin on egg quality, serum traits and antioxidant activity of laying hens. *Animal Feed Science and Technology, 163*(1), 59-66.

Toghyani, M., Toghyani, M., Gheisari, A., Ghalamkari, G., & Mohammadrezaei, M. (2010). Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). *Livestock Science, 129*(1), 173-178.

Trindade, J. L., Nascimento, J. W. B., & Furtado, D. A. (2007). Qualidade do ovo de galinhas poedeiras criadas em galpões no semi-árido paraibano. *Revista Brasileira de Engenharia Agrícola e Ambiental, 11*(6), 652-657.

Valero, M. V., Farias, M. S., Zawadzki, F., Prado, R. M., Fugita, C. A., & Rivaroli, D. C. (2016). Feeding propolis or functional oils (cashew and castor oils) to bulls: performance, digestibility and blood cells counts. *Revista Colombiana de Ciencias Pecuarias,* 29(1), 33-42.

Valero, M. V., Prado, R. M., Zawadzki, F., Eiras, C. E., Madrona, G. S., & Prado, I. N. (2014). Propolis and essential oils additives in the diets improved animal performance and feed efficiency of bulls finished in feedlot. *Acta Scientiarum. Animal Sciences, 36*(4), 419-426.

Received on March, 30, 2016.
Accepted on July, 5, 2016.