ISSN 1682-8356
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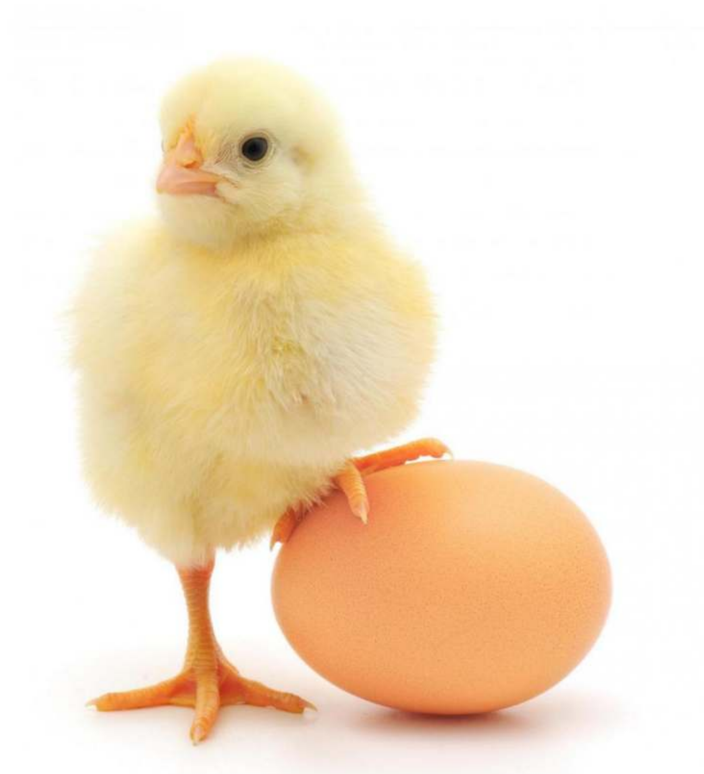

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 12 (11): 647-652, 2013
ISSN 1682-8356

Bioefficacy of the Copaiba Oil (*Copaifera* sp.) in Diets of Laying Hens in the Second Production Cycle in Humid Tropical Climate

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Abstract: The objective of this study was to evaluate the effect of supplementation of crescent levels (0, 0.10, 0.15, 0.20, 0.25 and 0.30%) of copaiba oil diets on performance, egg quality, biometrics intestines and resistance of the tibia of laying hens in the second production cycle. The experiment lasted 84 days with four intervals of 21 days each. Were used 144 layers Hissex White to 88 weeks of age and were housed in cages 24 used. The experimental design was completely randomized with six treatments and four replications of six birds each. Estimates of the levels of copaiba oil were determined by polynomial regression. The variables feed intake, egg production, feed conversion per dozen eggs, feed conversion per egg mass and egg mass were not significantly influenced (p>0.05) by treatments. The specific gravity of the egg and the percentage of egg shell showed a quadratic effect, getting his best result at the level of 0.15% inclusion of copaiba. The weight of the cecum showed a quadratic effect with his best result in the inclusion of 0.25%. Copaiba oil can be used as an additive in at 0.30% of diets of laying hens. As increased by the inclusion of the oil feed improved the percentage of shell and specific gravity, which may indicate possible relationship of copaiba oil with calcium metabolism.

Key words: Additive, productive performance, egg shell

INTRODUCTION

The poultry has evolved significantly over the years as important in the production of human food of high biological value segment, adapting to the techniques that allow the improvement of efficiency of poultry production in the shortest time.

Feeding laying hens still represents the largest fraction of the cost of production and small improvements in the efficiency of utilization of the nutrients can result in large economies (Vieira, 2001), thereby stimulating the poultry industry as a whole. Though the proven benefits of antibiotic additives on the health and welfare of the birds in conditions of health and management (Roura *et al*., 1992), there are disagreements about the increased metabolic efficiency as a function of the nutrients contained in diets with these additives.

One of the alternatives in evidence for the use of synthetic antibiotics in poultry feed is the copaiba oil (*Copaifera* sp.), known as Amazon balm due to its anti-inflammatory, antibacterial and antibiotic properties, beyond assist in metabolic regulation (Biavatti *et al*., 2006). This oil is extracted from the trunk, the branches and leaves of the copal, which is characterized as a large tree, reaching up to 36 m tall.

Copaiba oil is rich in terpenes (monoterpenes and sesquiterpenes) compounds found in oils from essence, extracted from vegetables and fruits. According to Estevão *et al*. (2009), copaiba oil is rich in sesquiterpenes oxygenated and non-oxygenated such as: α-cubebeno, α-cupaeno, caryophyllene and humulene. Both monoterpenes as the sesquiterpenes, act as anti-inflamatories, anti-infectives, analgesics and expectorants, agreeing with Vieira (1992), Lima *et al*. (2003) and Santos *et al*. (2008), which have proven these properties of activity of copaiba oil.

Vegetable oils are usually added to poultry feed in order to improve the palatability and feed conversion, in addition to reducing the loss of nutrients and optimize the energy intake of these. For birds in the second production cycle, it is observed mainly the nutritional aspect in order to ensure a greater extension of the production of these.

In Brazil, much of the laying breeding is subjected to forced molting, a practice that principally aims at improving production rates and egg quality of laying the end of the production cycle. However, the nutrition in this second production cycle is still poorly studied (FASSANI, 2002). In the creations of laying hens, the forced molting is usually adopted in situations of economic crisis as a way to postpone, for 25 to 30 weeks, the replacement of the lot. It is a practice that aims to extend the economic life of the birds at the end of the 1st cycle of production, causing the reproductive system of the bird rest for a period in order to regenerate the production capacity,

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improving shell quality and reducing the level of loss eggs (RAMOS *et al*., 1999). Seeking its extensive potential use, this study aimed to evaluate the supplementation of different levels of copaiba (*Copaifera* sp.) in feed for laying hens in the second production cycle through the productive performance, egg quality, biometrics of the intestines and the resistance of tibia.

MATERIALS AND METHODS

The experiment was conducted at the premises in the Sector of Poultry of the Department of Animal and Vegetable Production (DPAV) of College of Agrarian Sciences (FCA) of Federal University of Amazonas (UFAM) located in the Southern Sector of the University Campus, Manaus, Amazonas, Brazil. According to Köppen, the climate is classified as humid tropical climate with average annual rainfall of 2,286 mm and average temperature between 27-29°C (Inmet, 2013).

The experimental aviary used measures 17.0 m long and 3.5 m wide, having galvanized wire cages, chute type feeders and drinkers nipple type. Were used 144 laying hens Hissex White to 88 weeks old, created under identical conditions of feeding and management. The birds were subjected to forced molting process before the start of the experimental period, with the restriction of food for ten days and water *at libitum* with 70 weeks of life.

The experimental period was from August to November of 2011, divided into four periods of 21 days. However, before this period, the birds were subjected to an adaptation period of seven days the experimental diets and facilities. All birds were weighed at the beginning of the experiment to standardize plots and had a mean weight of 1.54±0.16 kg. Egg collection was performed three times a day (at 8, 11 and 16 h) and then recorded each occurrence. The birds were distributed in a completely randomized design, consisting of six treatments (0, 0.1, 0.15, 0.2, 0.25 and 0.3% supplementation of copaiba oil in feeds) and four replicates of six birds each. During the experimental period was provided 16 hours of light/day (natural+artificial).

Copaiba oil was obtained from extractive trade and the product extracted from fruits of copaibeiras located in the district of Manicoré, distant 330 km from Manaus, the capital of Amazonas state, Brazil. The extraction process consisted of the rational method of extraction where there will be a small hole in the trunk of the tree looking to reach the shaft, sealing the canal after extraction. After extraction, the oil-resin was strained and thus obtained virgin oil, maintaining good level of purity.

The nutritional requirements of the birds were cared according by the breed manual used (Globoaves, 2006). The isonutritives diets (2750 kcal ME/kg, 15.5% CP, 4% Calcium and 0.45% Phosphorus) were formulated according to the values of ingredients provided by the Brazilian Tables for Poultry and Swine (Rostagno *et al*., 2011), except for the composition of the copaiba and are in Table 1.

The performance of birds were evaluated: feed intake (g/bird/day), egg production (%), feed conversion (kg feed/kg egg mass) and feed conversion (kg feed per dozen eggs). In the last two days of each evaluation period (21 days), were collected 24 eggs from each treatment (four eggs per replicate) for analysis of egg quality, where were evaluated: egg weight (g), egg mass (g), albumen weight (g), yolk weight (g), albumen height (mm), yolk height (mm), shell weight (g), shell thickness (μm), specific gravity (g/cm^3) and yolk color. Before undergoing the assessment, eggs were stored for one hour in order to equalize its temperature to room temperature.

The eggs were weighed out on an electronic scale to the nearest 0.01 g. The egg mass was obtained by calculating the quotient between weight eggs and egg production multiplied by one hundred. Whole eggs after weighing were placed in wire baskets and immersed in plastic buckets containing different levels of sodium chloride (NaCl), from the lowest to the highest concentration, with variations in density from 1.075 to 1.100 g/cm^3, with an interval 0.005 between them. Eggs were removed from the float to the surface with their, respective values noted.

For the analysis of the weight of albumen and yolk, were used a manual albumen and yolk separator. The albumen and yolk were placed in plastic cups, both weight weighed on an analytical balance and weighed. To calculate height of albumen and yolk, they were placed on a flat plate to measure their values. The procedure for determining the height of albumen and yolk is to measure at the middle region between the outer edge of the albumen and yolk. Were used to measure the heights an electronic caliper, with the values recorded in millimeters. The weight of the egg shell was obtained after the same were washed, dried at ambient temperature for 48 h and then weighed in grams.

The husks and his reading made with the aid of a micrometer were used to determine the thickness of the shell. Data were collected in three regions of the shell: basal, equatorial and apical and the values were recorded. From the values obtained in the three regions were calculated the average, in micrometers, the thickness of the eggshell. To evaluate the pigmentation of egg yolk, were used the Roche color fan with points 1 to 15. To determine the Haugh unit used the formula proposed by Silva (2004).

In the analysis of biometrics of the intestines were sacrificed by cervical dislocation 24 birds, four birds per treatment. In order to analyze the capacity of absorption of nutrients were separated the small intestine (duodenum and jejunum+ileum) and large intestine

Table 1: Composition of experimental diets containing copaiba oil

	Levels of copaiba (%)					
	0	1	2	3	4	5
Ingredients	I	II	III	IV	V	VI
Corn	68.22	68.09	68.03	68.11	67.91	67.85
Soybean Meal	18.1	18.12	18.13	18.14	18.15	18.16
Calcareous	8.49	8.49	8.49	8.49	8.49	8.49
Meat Meal-45% CP	3.00	3.00	3.00	2.85	3.00	3.00
Bicalcic phosphate	1.20	1.20	1.20	1.20	1.20	1.20
Premix Vit. Min.[1]	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.35	0.35	0.35	0.35	0.35	0.35
DL- Met 99	0.14	0.14	0.14	0.15	0.14	0.14
Copaiba oil	-	0.10	0.15	0.20	0.25	0.30
Butil-Hidroxi-Tolueno (BHT)[2]	-	0.01	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient						
Metabol Energy, kcal^{-1}/kg	2.750	2.750	2. 750	2. 750	2. 750	2. 750
Crude Protein, (%)	15.500	15.500	15.500	15.500	15.500	15.500
Methionine+Cystine, (%)	0.656	0.656	0.656	0.656	0.656	0.56
Methionine, (%)	0.395	0.395	0.395	0.395	0.395	0.95
Calcium, (%)	4.000	4.000	4.000	4.000	4.000	4.000
Available phosphorus, (%)	0.450	0.450	0.450	0.450	0.450	0.450
Sodium, (%)	0.185	0.185	0.185	0.185	0.185	0.185

[1]Assurance levels per kilogram of product:

Vitamin A 2.000.000 UI	Vitamin D3 400.000 UI	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 2000 mg	Folic Acid 50 mg	Cocciostatic 12.000 mg
Hill 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

[2]Antioxidant

(cecum, colon and rectum) of the other internal organs. Precision scale was used for each portion of the intestines to measure the weight and a millimeter ruler to measure the length of the intestines. For tests with the tibia bone have been selected only the left side bones of the birds. The tibia was cleaned by removing the muscle, cartilage or nerves. The bones were boiled for removal of waste and muscle were subsequently dried at ambient temperature. The analysis was performed with the aid of a mechanical equipment (EMIC 19) by applying a certain force, which is variable according to the body resistance and values recorded. But, before the analysis of resistance, the bones were measured with a ruler to determine the diameter thereof.

Statistical analysis was performed by computer program Statistical Analysis System-SAS (2008) and estimates of the treatments were subjected to analysis to polynomial regression in levels of 0.01 and 0.05 probability.

RESULTS AND DISCUSSION

The average results for the variables related to the productive performance are presented in Table 2. The performance variables of the birds showed no significant differences ($p>0.05$) between the means from the addition of different levels of copaiba oil in the diets. The results corroborate feed intake found for Faitarone (2010) to provide feed supplemented with flaxseed oil, canola oil, soybean oil and your combinations of layers also did not achieve significant changes in food intake. The feed conversions (kg/kg) and (kg/dz) worsened as increased the level of copaiba oil in the diets. The results of egg mass and percentage of production reduced to 0.15% of the level of addition of copaiba oil. The correlation found between increased feed intake and consequent worsening of feed conversion with the fall in production and egg mass may be associated with high fat content found in the properties of copaiba oil and its effect on the deposition of the adipose tissue These results differ from those obtained by Costa *et al.* (2008), where they studied different levels of canola oil and soybeans had found significant differences ($p>0.05$) on productive performance.

The average results obtained for the variables related to egg quality are showed in Table 3. No significant differences ($p>0.05$) were found between the means for egg weight, percentage of yolk and percentage of albumen.

In percentage of shell were found significant difference ($p<0.05$) between the treatments, observing quadratic effect ($Y = -0.0721x^2+0.53x+5.53$, $R^2 = 0.24$) and from the derivation this function was possible to estimate the maximum point (9.91%) on the growth curve.

The specific gravity was significantly influenced ($p<0.01$) the levels of copaiba oil, observing quadratic effect ($Y = -0.0004\ x^2+0.0028\ x+1.08$, $R^2 = 0.21$) where the derivation of the equation estimated the maximum point

Table 2: Feed intake, feed conversion kilo of feed per dozen eggs produced (kg/dz), feed conversion kilo of feed per egg mass produced (kg/kg), egg mass and percentage of production of laying hens in second cycle of production fed diets containing different levels of copaiba oil

	Inclusion levels of copaiba oil (%)							
	0	0.10	0.15	0.20	0.25	0.30	R^2	CV, (%)
Feed intake, g/bird/day	80.62	80.12	83.64	81.29	81.19	83.91	-	5.86
Feed conversion, kg/dz	1.33	1.35	1.49	1.40	1.50	1.52	-	9.95
Feed conversion, kg/kg	1.68	1.73	1.88	1.79	1.89	2.00	-	10.89
Egg mass, g $bird^{-1}day^{-1}$	62.36	61.24	58.43	58.57	55.85	56.70	-	10.07
Production, (%)	74.35	74.00	70.13	71.42	67.06	69.69	-	8.44

CV-Coefficient of variation

Table 3: Egg weight, percentage of albumen, percentage of yolk, percentage of shell, shell thickness, specific gravity and yolk pigmentation of eggs come from laying hens in second cycle of production fed diets containing different levels of copaiba oil

	Inclusion levels of copaiba oil (%)							
	0	0.10	0.15	0.20	0.25	0.30	R^2	CV, (%)
Egg weight, (g)	66.42	65.51	66.20	65.20	66.01	64.12	-	2.85
Percentage of albumen, (%)	61.03	58.49	57.91	58.68	59.02	57.61	-	4.55
Percentage of yolk, (%)	25.86	25.84	27.16	26.45	25.73	26.93	-	2.99
Percentage of shell,* (%)	9.10^{b}	9.25^{b}	10.27^{a}	9.92^{ab}	9.48^{ab}	9.70^{ab}	0.24	5.01
Shell thickness, µm	35.33	38.98	37.43	34.77	35.35	35.32	-	8.18
Specific gravity,** g/mL	1083.2^{b}	1084.2^{b}	1088.2^{a}	1084.0^{b}	1084.6^{ab}	1083.8^{b}	0.21	0.15
Yolk pigmentation	5.64	5.45	5.87	6.01	6.09	6.28	-	0.30

CV-Coefficient of variation, *Quadratic effect ($p<0.05$), **Quadratic effect ($p<0.01$)

(1.081 g/mL water) on the growth curve. The inclusion level of 0.15% copaiba oil showed higher specific gravity possibly indicating that a relationship exists between the copaiba oil and calcium metabolism in the formation of the eggshell because, after this, the averages presented linear growth and decreasing inversely proportional to the levels of these inclusion.

No significant difference ($p>0.05$) were found for shell thickness, a result with those found for the other variables related to the egg shell indicates that, regardless of birds have been forced molting and were fed diets containing different levels of copaiba oil, the quality of the standard shell, determined by these variables did not change according to Peebles and McDaniel (2004).

No were found significant differences ($p>0.05$) for yolk color, having the means showed behavior linearly increased as the level of inclusion of copaiba oil in the diets. According Leandro (2012), oil-resin of copaiba has one hundred sesquiterpenes and thirty seven diterpenes, which characterizes the natural biological factor pigmentation of this.

The average results obtained for the variables related to the length of the small and large intestines of birds are shown in Table 4. No significant differences ($p>0.05$) were found for the variables related to intestinal length of birds. The relative values corroborate with those found by Franzo *et al.* (2006) that studying different levels of forced molting in laying hens lineage Hisex Browm, found no significant results in the length of the intestines.

Observed decrease in the duodenal length from the inclusion of copaiba oil in the diets. However, the length of the small intestine have a proportional increase with increasing the levels. Both behave as intermittent growth between the means, being the lowest value found for the duodenum in the highest level of inclusion as to the jejunum+ileum the highest value was obtained at the highest level of inclusion. Results also observed for length of cecum and colon+rectum, where growth was also intermittently between means. The functional element in the small intestine as the mucosa, which can be characterized as a nutrient permeable layer and barrier against harmful compounds. The competition between bacteria and host for nutrients and the formation of metabolites depressants growth in the intestine may have negative effects on the mucosa of the small intestine. It is believed that the inhibition of microbial activity by antibiotics reduces competition with the host for the nutrients and the production of metabolites for growth depressants (Van Leeuwen, 2002).

The average results for the variables related to the weight of the small and large intestines of birds are presented in Table 5. The weight of the cecum showed a significant difference ($p<0.05$) between treatments, showed a quadratic effect ($Y = -0.0102 x^2+0.26 x+6.54$, $R^2 = 0.07$) for the inclusion levels of oil copaiba, where from derivation of this function was possible to estimate the point of maximum curvature (4.88%). The inclusion level of 0.25% showed greater weight of the cecum, however, observed similar behavior for the media in

Table 4: Length of the duodenum, jejunum+ileum, cecum e colon+rectum of laying hens in second cycle of production fed diets containing different levels of copaiba oil

	Inclusion levels of copaiba oil (%)							
	0	0.10	0.15	0.20	0.25	0.30	R^2	CV, (%)
Duodenum, cm	9.09	8.90	8.11	8.34	8.30	8.00	-	10.37
Jejunum+ileum, cm	37.33	36.90	38.15	37.76	37.68	38.03	-	2.78
Cecum, cm	4.66	5.14	4.70	4.94	5.29	5.12	-	10.37
Colon+rectum, cm	2.72	3.25	2.76	2.89	2.72	3.14	-	18.31

CV-Coefficient of variation

Table 5: Weight of the duodenum, jejunum+ileum, cecum and colon+rectum of laying hens in second cycle of production fed diets containing different levels of copaiba oil

	Inclusion levels of copaiba oil (%)							
	0	0.10	0.15	0.20	0.25	0.30	R^2	CV, (%)
Duodenum, g	27.09	22.00	23.49	24.95	23.74	21.92	-	15.21
Jejunum+ileum, g	49.24	55.02	57.11	52.46	54.22	55.68	-	8.23
Cecum, g*	7.60^{abc}	5.87^{c}	6.57^{bc}	8.29^{ab}	8.46^{a}	7.04^{abc}	0.07	11.31
Colon+rectum, g	14.55	17.10	15.31	16.79	16.56	15.33	-	16.63

CV-Coefficient of variation, *Quadratic effect ($p<0.05$)

Table 6: Diameter measures and resistance to flexion-compression of the left tibia of laying hens in second cycle of production fed diets containing different levels of copaiba oil

Inclusion levels of copaiba oil (%)	Resistance (Kgf)	Diameter (cm)
0	10.69	6.13
0.10	11.07	6.20
0.15	13.84	6.47
0.20	11.84	6.30
0.25	11.99	6.50
0.30	12.99	6.18
CV (%)	15.243	---

CV-Coefficient of variation

relation to basal diet. The caeca of the birds promote digestion of amino acids by cecal microflora (Mortensen, 1984) and degradation of proteins by bacteria present therein (Chaplin, 1989). The other variables related to the weight of the intestines showed no significant differences ($p>0.05$) between the means.

The average results obtained for resistance and diameter of the left tibia of birds are shown in Table 6. There were no significant difference ($p>0.05$) in diameter and resistance of the left tibia flexion-compression and hence in bone structure. The greater bone diameter (6.50 cm) was obtained in inclusion level of 0.30% of copaiba oil in the diets, while the greater bone strength (13,84 kgf) in the inclusion of 0.15%. The results differ from Murakami *et al.* (2009) that assessed the effects of flaxseed oil to the diet on the parameter of bone quality of broilers and it was found that this presents a significant difference in the growth and mineralization of the long bones. The addition of vegetable oils as the fat source may have influence on calcium metabolism in the body of the poultry.

Conclusion: Some conclusions were determinate starting from the found results. Copaiba oil can be used as an additive to level of 0.30% in the diets of laying hens in second cycle of production. As from increased levels of inclusion of copaiba oil in feed the percentage of shell and specific gravity improved, which can indicate possible relationship of copaiba oil with calcium metabolism.

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