USE OF NATURAL PIGMENTATION BASED ON ANNATTO AND PAPRIKA IN THE DIET OF COMMERCIAL LAYING HENS

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Resumo: This experiment was conducted to evaluate the effects of the inclusion of a natural pigment composed of the oily extract of annatto (Bixa orellana L.) and paprika oleoresin (Capsicum annum) on productive performance, egg quality, and effect on egg storage in 440 laying hens from 34 to 46-wks distributed in randomized design with five treatments, 11 replicates of eight birds each. The treatments consisted of a control diet (0% - T1) and four diets with the natural pigment (0.015% - T2, 0.03% - T3, 0.045% - T4 and 0.06% - T5). The experimental period was three cycles of 28 days each. The parameters of feed intake, egg production, egg mass, feed conversion per dozen and feed conversion per egg mass, as well as weight, albumen height, yolk color, Haugh unit, strength and shell thickness and the effect on storage were evaluated at the end of each cycle. There was a significant difference between the increase in the pigmentation of the yolk and the increase in albumen height, related to the addition of the natural pigment and this one remained despite the time. The better results were when we used 0.06 % of the natural pigment showing an increase in egg yolk pigmentation, but we observed that visual difference for the consumer's eyes was not so striking.
Palavras Chave: Egg quality; oily extract; oleoresin; storage.

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Abstract: This experiment was conducted to evaluate the effects of the inclusion of a natural pigment composed of the oily extract of annatto (Bixa orellana L.) and paprika oleoresin (Capsicum annum) on productive performance, egg quality, and effect on egg storage in 440 laying hens from 34 to 46-wks distributed in randomized design with five treatments, 11 replicates of eight birds each. The treatments consisted of a control diet (0% - T1) and four diets with the natural pigment (0.015% - T2, 0.03% - T3, 0.045% - T4 and 0.06% - T5). The experimental period was three cycles of 28 days each. The parameters of feed intake, egg production, egg mass, feed conversion per dozen and feed conversion per egg mass, as well as weight, albumen height, yolk color, Haugh unit, strength and shell thickness and the effect on storage were evaluated at the end of each cycle. There was a significant difference between the increase in the pigmentation of the yolk and the increase in albumen height, related to the addition of the natural pigment and this one remained despite the time. The better results were when we used 0.06 % of the natural pigment showing an increase in egg yolk pigmentation, but we observed that visual difference for the consumer's eyes was not so striking.
Keywords: Egg quality; oily extract; oleoresin; storage.

Introdução: The egg is a food that has highly nutritious qualities and is considered one of the most important protein needs for the world population (Magdelaine, 2011). Color is one of the factors that influences the decision of the consumer when purchasing a product; associated to the quality and nutritional value of the food (Coutts and Wilson, 2007).Due to growing concern about food quality and banning the addition of synthetic pigments to animal feed, the natural pigments have been used in the food industry (Yabiku, 1992). Carotenoid became instable with storage and when they go through heating processes (Minguez-Mosquera et al., 1996).Microencapsulation is one of the techniques for preserving the quality of sensitive substances protects them from the external environment (Ghosh, 2006).This study aims to show the effect of a microencapsulated powder of oily extract of annatto and oleoresin of paprika in laying hens feed on the interference of quality of eggs, and the effect on the storage.

Material e Métodos: A total of 440 laying hens (34-wk old) were distributed in a randomized design, with five treatments and 11 replicate. The treatments consisted of a control group T1 and the levels of inclusion of the pigment: 0.015% - T2; 0.03% - T3; 0.045% - T4 and 0.06% - T5 / ton. The experimental period totaled three cycles of 28 days each. The hens were fed a corn-soybean meal basal diet (Table 1) which was based on nutritional levels formulated according to Rostagno et al., 2017. A premixing was performed with one kilo of corn before mixing with the rest of the diet to obtain a better homogenization. The eggs were collected daily, specifying: egg production, quantity of intact eggs, unviable (broken, cracked), deformed and dirty eggs. Feed intake, egg production, egg weight, egg mass and feed conversion parameters were determined at the end of each 28-day cycle. On day 27 and 28, of each cycle, two eggs were collected per replicate per day, totalizing four eggs and analyzed individually by the Digital Egg Tester (DET6000). The evaluated characteristics of egg quality were: weight, albumen height, yolk color, Haugh unit, strength and shell thickness. In addition, two eggs were collected the last two days at the end of each cycle and stored for 21 days, to analysis the permanence of pigmentation mainly. The effect of the pigment levels on characteristics of performance, internal and external egg quality was evaluated by multiple regression analysis, through the computer program Stata 15.1 (2017). Differences were considered significant at the level of $P < 0.05$.

Resultado e Discussão: There were no significant differences observed for feed consumption, feed conversion or egg-laying performance (Table 2) related to inclusion of the natural pigment in the diet, except for egg yolk color and albumen height (Table 3) These results were similar to those found in Yamauchi et al., 2011, where they used paprika combined with a probiotic to enhance egg yolk color. The only characteristic that differed from these results was that the albumen height was statistically significant. However, in this experiment, the r squared for this variable was low (R2=0.18), which means that the independent variable does not explain much of the variability in the dependent variable. There is a lack of information about the relationship between these two variables, showing that these results could contribute to further studies. Silva et al., 2006 partially replaced the maize by sorghum in and added annatto seed residue, but the egg yolk color score was higher than this experiment, this can be related to the concentration level of the Annatto in the form of an oily extract. The parameter pigmentation of yolk was affected by the increase in consumption of the natural pigment in the feed as shown by the regression equation (Y=4.036364+0.0086061X; R2 = 0.72) in our statistical analysis.The statistical analysis for our storage eggs for 21 days and the characteristic of yolk color showed a highest increase of the color with a long period of storage with a better R2 (Table 3) on the contrary of Harder et al., 2005 which observed a lower color of the yolk.

Table 1. Ingredient composition and nutrient of the basal diet provided between the 34 to 46 weeks of age of the hens with the pigment levels.

Ingredients	%
Corn	52.78
Soybean meal	30.50
Limestone	9.04
Soybean oil	5.03
Dicalcium phosphate	1.67
Salt	0.43
Vitamin and mineral supplement *	0.40
DL-methionine	0.19
Total	100.00
Calculated analysis (% as fed)	
ME (kcal/kg)	2.950
Crude protein	18.50
Calcium	4.00
Available phosphorus	0.41
Digestible lysine	0.88
Sulfur amino acids (AAS)	0.70
Digestible Methionine	0.45
Digestible Threonine	0.62
Sodium	0.21

*Provided per kg of diet: Iron (min.) 0.04 g/kg; Copper (min.) 10 mg/ kg; Manganese (min.) 0.08 g/kg; Zinc (min.) 0.1 g/kg; Iodine (min.) 0.832 mg/kg; Selenium (min.) 0.3 mg/kg; Vitamin A (min.) 7.000,00 UI/kg; Vitamin D3 (min.) 2.500,00 UI/kg; Vitamin E (min.) 8.00 UI/kg; Vitamin K3 (min.) 1.58 mg/kg; Vitamin B1 (min.) 1.00 mg/kg; Vitamin B2 (min.) 4.00 mg/kg; Niacin (min.) 20.1 mg/kg; Pantothenic acid (min.) 7.22 mg/kg; Vitamin B6 (min.) 1.00 mg/kg; Folic acid (min.) 0.297 mg/kg; Biotin (min.) 0.02 mg/kg; Vitamin B12 (min.) 9.6 mcg/kg; Choline (min.) 0.3 g/kg; Methionine (min.) 0.7 g/kg; Halquinol 30 mg/kg

Table 2. Albumen height (AH), Yolk color (YC), Shell thickness (SHT), Weight (W), Strength thickness (STT), Haugh unit (HU), Yolk color storage at 21 days (YCS) of laying hens eggs, fed with different levels of natural pigment.

	AH(mm)	YC	SHT(mm)	W(g)	STT(kgf)	HU	YCS
Levels (%)			first	cycle			
0.00	6.98	3.70	0.41	59.19	4.80	82.46	3.82
0.015	7.18	3.86	0.41	59.09	4.46	83.33	4.09
0.030	7.54	4.23	0.39	61.31	4.48	86.33	4.66
0.045	7.45	4.45	0.39	61.61	4.61	85.36	4.73
0.060	8.02	4.36	0.41	62.10	4.48	88.74	4.75
P value	0.001	0.001	0.307	0.001	0.750	0.003	<0.001
Regression	**	**	NS	**	NS	**	**
Levels (%)			second	cycle			
0.00	7.46	4.32	0.43	63.29	4.13	84.26	4.82
0.015	7.37	4.75	0.43	62.64	4.49	84.27	4.93
0.030	7.61	4.98	0.44	63.82	4.56	86.17	5.45
0.045	7.63	5.26	0.43	65.14	4.63	85.43	5.91
0.060	8.11	5.62	0.42	62.90	4.49	88.56	6.07
P value	0.05	0.001	0.121	0.402	0.022	0.063	<0.001
Regression	**	**	NS	NS	**	NS	**
Levels (%)			third	cycle			
0.00	7.52	4.05	0.40	64.68	4.30	85.28	4.66
0.015	7.53	4.34	0.40	63.62	4.14	85.69	4.95
0.030	7.33	4.73	0.39	64.58	4.22	84.03	5.00
0.045	7.85	4.93	0.40	64.60	4.32	86.99	5.73
0.060	7.85	5.36	0.39	65.35	3.76	86.85	5.98
P value	0.043	0.001	0.037	0.331	0.018	0.103	<0.001
Regression	*	**	**	NS	**	NS	**

NS = not significant.
** = the value of the R^2 was very low, they were not considered for the present study.

*Y=6.966727 + 0.0062885X; R^2 = 0.18
*Y=3.740909+0.0050909X; R^2 = 0.20
*Y=3.909091+0.0066667X; R^2 =0.39
*Y=7.327818+0.0041455X; R^2 =0.06
*Y=4.381818+0.0077576X; R^2 =0.55
*Y=4.740909 +0.0092727X; R^2 =0.59
*Y=7.422364+0.0026265X; R^2 =0.07
*Y=4.036364+ 0.0086061X; R^2 = 0.72
**Y=4.581818+0.0090909X; R^2 = 0.72

Table 3. In this table we can observe the performance parameters and the P value in all the treatments with the respective average of all those parameters that were evaluated. Egg production (EO), Feed consumption (FC), Mass of eggs (ME), Conversion per dozen (CD), Conversion per egg mass (CM).

	EP (%)	FC (g bird/day)	ME (g)	CD (kg/dz)	CD (kg/kg)
Levels (%)			first	cycle	
0.00	97.24	90.96	58.28	1.12	1.56
0.015	98.05	93.46	59.02	1.14	1.59
0.030	97.61	90.28	59.83	1.11	1.51
0.045	96.38	92.45	60.95	1.13	1.52
0.060	98.86	91.70	61.24	1.11	1.50
P value	0.057	0.902	0.016	0.442	0.051
Regression	NS	NS	**	NS	NS
Levels (%)			second	cycle	
0.00	97.39	94.89	60.18	1.17	1.57
0.015	98.05	93.55	62.03	1.15	1.51
0.030	96.06	97.39	60.76	1.22	1.60
0.045	9.91	97.18	61.44	1.19	1.58
0.060	98.46	97.18	61.58	1.19	1.58
P value	0.445	0.083	0.340	0.157	0.283
Regression	NS	NS	NS	NS	NS
Levels (%)			third	cycle	
0.00	96.46	92.16	60.37	1.15	1.53
0.015	97.28	93	61.37	1.15	1.52
0.030	95.45	96.68	59.87	1.21	1.61
0.045	98.10	95.42	61.60	1.17	1.55
0.060	97.50	94.69	60.25	1.17	1.57
P value	0.276	0.172	0.999	0.465	0.229
Regression	NS	NS	NS	NS	NS

NS = not significant
** = the value of the R^2 was very low, for which reason they were not considered for the present study.

Conclusão: The use of the natural pigment showed an increase in egg yolk coloration, but compared with the results of other studies, we observed greater yolk pigmentation. The effect on storage of 21 days was maintained, showing good sensory characteristics for the consumers. The dose of the natural pigment could be increased, but the profitability of the product would have to be analyzed.

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