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Egg quality of laying hens fed diets with plant extracts

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ABSTRACT. The objective of this study was to evaluate the effect of marigold flower extract and paprika on egg quality of Black Avifran laying hens in different periods of storage. A completely randomized design study with a 3x3 factorial scheme with three diets (control, paprika, and marigold flower extract) and three storage periods (0, 7, and 14 days) was carried out. The following metrics were evaluated: egg weight, yolk weight, shell weight; yolk color, albumen height, specific gravity, and Haugh unit as well as the yolk, shell, and albumen percentage relative to the total weight of the egg. There was no significant interaction between additives and storage time ($p > 0.05$) for most of the parameters evaluated. The only exception was observed for yolk color, in which the interaction effect between treatments was significant ($p < 0.05$). The effect of storage time was significant ($p < 0.05$) for albumen weight, albumen percentage, yolk color, albumen height, specific gravity, and Haugh unit, with reduction of these parameters over the evaluation period. The use of paprika and marigold flower extract in the diets of Black Avifran laying hens does not influence egg quality.

Keywords: antioxidants; egg quality; shelf life.

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Introduction

The physical or sensorial quality of eggs can be defined as a set of characteristics that are responsible for their acceptability in the market. The egg is a perishable food and it begins to lose its internal quality immediately after it is laid (Berardinelli, Donati, Giunchi, Guarnieri, & Ragni, 2003). The storage time, egg temperature, poultry strain, and age, as well as the nutritional management and sanitary status of laying hens are factors that influence egg quality (Harder, Brazaca, Savino, & Coelho, 2008). Therefore, it is necessary to find ways to maximize egg production and its shelf life.

Egg farms have looked for alternatives to increase shelf life of eggs and decrease the deterioration of the product. The use of antioxidants appears to be a reliable option, since they increase the shelf life of eggs intended for consumers (Kovacs-Nolan, Phillips, & Mine, 2005). Most of the research on the use of antioxidants has been carried out with the addition of synthetic products. However, the use of these additives has been prohibited in Major European and North American countries, which in turn increased the need for other alternatives such as plant extracts.

Supplementary plant extracts as an animal feed are categorized as phytogenic additives (Berardinelli et al., 2003). These additives can be defined as plant-derived compounds that are incorporated into the animal diets in order to increase animal performance and the quality of the products obtained (Santana, Oliveira, Barnabé, Mendes, & Andrade, 2011). Bonato et al. (2009) report that plant extracts have shown antimicrobial capacity, but their influence on egg quality have not yet been studied. Some of these extracts may improve the digestibility of the animals, as they increase the palatability of the diet, stimulate the secretion of enzymes, and benefit the intestinal microflora, resulting in a better nutritional deposition in the eggs.

Urucum extract (*Bixa orellana*), saffron (*Curcuma longa*), marigold flower (*Tagetes erecta*), and paprika (*Capsicum annum*) are the natural extracts mostly used in animal feeding (Moura et al., 2011). They are frequently used to confer pigmentation to the egg yolk, but little is known about their antioxidant activity.

Therefore, the objective of the study was to evaluate the effect of marigold flower and paprika added to the diet of Black Avifran animals as well as storage time on egg quality.

Material and methods

The experiment was carried out in the poultry sector of the Experimental Farm of the Department of Animal Science and Rural Extension of the Federal University of Mato Grosso (UFMT). The Ethics Committee on the Use of Animals from the UFMT approved the experimental procedures used in this study (Protocol nº 23108.092960/2015-80). A completely randomized design with a 3 x 3 factorial scheme (three diets and three storage periods), with 10 replicates was used. Three eggs from each plot were considered the experimental replicate. We evaluated three storage periods: zero (fresh egg), seven, and 14 days of storage as well as two plant extracts and one control (without plant extract). The extracts used were commercially acquired. We used marigold flower (*Tagetes erectus*) and paprika (*Capsicum annuum*).

Ninety Black Avifran laying hens of 60 weeks old were housed in the experimental barn for 63 days during the pre – experimental phase. Eighteen experimental boxes were used for each treatment and split between the three types of diets (control, marigold flower, and paprika) with five birds in each box. Animals were raised in the floor system, with a nest (0.6 mouths $bird^{-1}$), one roosting bar, and bedding of rice husk. The boxes measured 1.76 x 1.53 m (length x width), providing 0.538 m^2 $bird^{-1}$. A light program of 16 hours day^{-1} was used. Feed and water were provided *ad libitum* to the animals. Daily maximum and minimum temperatures as well as relative humidity were recorded using thermos-hygrometers placed inside the boxes.

The quantity of extracts added to the diets was those indicated by the manufacturer as follows: 0.8% of paprika (*Capsicum annuum*) and 0.8% of marigold flower extract (*Tagetes erectus*). The marigold flower extract contained 12 g kg^{-1} of xanthophylls from which 80 to 90% was lutein. The paprika contained about 4 to 8 g kg^{-1} of xanthophylls from which 50 to 70% were capsanthin. The diets (Table 1) used in the experiment were formulated to be isocaloric (2775 kcal kg^{-1}), isoproteic (17.7% of crude protein), isophosphoric (0.46% of phosphorus), and isocalcitic (3.9% of calcium) according to the requirements established by Rostagno et al. (2011) for semi-heavy birds.

Fresh eggs were collected from each treatment and stored in cardboard trays suitable for eggs according to the day of collection. These were stored in an enclosed room with no solar incidence and with average air temperature and humidity of 28.2°C and 58.35%, respectively. The following metrics were evaluated: egg weight (g; EW), yolk weight (g; YW), shell weight (g; SW); yolk color, albumen height (mm; AH), specific gravity, and Haugh unit (HU) as well as the yolk, shell, and albumen percentage relative to the total weight of the egg. Eggs collected on each day were identified and weighed individually in an analytical balance with an accuracy of 0.001 g.

Table 1. Experimental diets and their calculated composition

Ingredients	Treatments		
	Control	Paprika	Marigold flower
Corn (%)	61.98	61.90	61.90
Soybean meal (%)	25.00	25.00	25.00
Soybean oil (%)	1.52	1.52	1.52
Limestone (%)	8.10	8.10	8.10
Sodium chloride (%)	0.50	0.50	0.50
Dicalcium phosphate (%)	1.10	1.10	1.10
Paprika (%)	0.00	0.08	0.00
Marigold flower (%)	0.00	0.00	0.08
Mineral-vitamin premix (%)[1]	1.80	1.80	1.80
Nutrients	Composition (%)		
Crude protein	17.7	17.7	17.7
Calcium	3.7	3.7	3.7
Available phosphorus	0.38	0.38	0.38
Total lysine	0.92	0.92	0.92
Total methionine + cysteine	0.71	0.71	0.71
Threonine	0.69	0.69	0.69
Metabolizable energy	2750	2750	2750

[1]The mineral-vitamin premix contained (per kg): 900.0 mg of folic acid; 12000.0 mg of pantothenic acid, 77.0 mg of biotin, 130.0 mg Ca, 4,0000.0 mg of Niacin, 370.0 mg Se, 8,800,000.0 IU vitamin A, 2,500.0 mg vitamin B1; 0.04 g growth vitamin, 0.02 g of antioxidant, 75.0 mg Mn, 50.0 mg Zn, 8.0 mg Cu; 0.75 mg I; 50 mg Fe, 7000.0 mg Co.

The specific gravity was determined by immersing the eggs in saline solutions with densities ranging from 1.070 to 1.095 g cm^{-3} with an interval of 0.005 g cm^{-3}. The solutions were prepared using a calibrated densimeter (OM-5565, Incoterm). Next, the eggs were broken for analyzes of albumen height and weight as well as the percentage of yolk, shell, and albumen. Albumen height was measured in its median region using a digital calliper (Starret 150 mm). The HU was obtained using formula proposed by Alleoni and Antunes (2001):

$$HU = 100 \times \ln\left\{AH - \frac{\sqrt{G \times \left(30 \times W^{0.37} - 100\right)}}{100} + 1.9\right\}$$

where AH is the albumen height (mm), G is the gravitational constant of 32; and W is the weight of the egg (g).

Egg yolks were placed on a light-color surface and its color was obtained by comparison with a Yolk Color Fan (DSM color palette). The yolk of each egg was weighed on an analytical balance with an accuracy of 0.001 g.

The weight of the albumen was estimated based on the difference between the total weight of the egg and the weight of the yolk plus the weight of the shell, which was obtained after it was rinsed and dried for 48 hours at room temperature.

The percentage of albumen, yolk, and shell were obtained by dividing the weights of each one of these components over the weight of the whole egg and the result multiplied by 100.

All evaluations were carried out by the same people and always in the same place and same luminosity in order to avoid variations.

Results were analyzed using two-way analysis of variance and means were compared using the Tukey test at 5% of probability using the statistical software. The analysis was done using the statistical program Assistat (Assis & Azevedo, 2016) and adopting the following statistical model:

$$Y_{ijk} = \mu + A_i + S_j + (AS)_{ij} + \varepsilon_{ijk}$$

where Yi is the observed value of dependent variable (egg weight, yolk weight, shell weight, etc), μ is the overall mean, Ai is the effect of the ith plant extract (control, paprika, and marigold flower), Sj is the effect of the jth storage time (0, 7, and 14 days), (AS)ij is the effect of the interaction between the ith plant extract and the jth storage time, and εijk is the residual error associated to the Yijk observation.

Results and discussion

There was no significant interaction between additives and storage time ($p > 0.05$) for most of the parameters evaluated. The only exception was observed for yolk color, in which the interaction effect between treatments was significant ($p < 0.05$). The effect of storage time was not significant ($p > 0.05$) for egg weight, yolk weight, shell weight, yolk percentage, and shell percentage (Table 2).

The storage time influenced the quality of the eggs ($p < 0.05$), decreasing the weight and the percentage of albumen, yolk color, albumen height, specific gravity, and HU. As the storage time increased, the percentage of albumen decreased. According to Kirunda and McKee (2000), factors influencing the resistance of the yolk membrane are the same that alter the quality of the albumen. Still, according to the same authors, the quality of the eggs is influenced by temperature, humidity, handling, and storage time.

Barbosa, Freitas, Sakomura, and Wada (2004) studied the effect of temperature and storage time on the internal quality of commercial eggs. They observed a linear increase in the percentage of egg yolk as a function of storage time. While working with paprika extract, Moreno et al. (2007) reported that egg quality was not affected except for the color of the yolk, which increased with the inclusion of paprika in the diets.

Ramos, Camargo, Oliveira, Cedro, and Morenz (2012) evaluated the effects of storage time on egg quality, and concluded that an increase in storage time, as well as storage at room temperature, promoted a change in the internal quality of the eggs, which resulted in decreased albumen weight.

Özek, Wellmann, Ertekin, and Tarım (2011) found an increase in albumen height and HU values in eggs from laying hens fed with a mixture of essential oils. These results were not observed in our study (Table 2).

Table 2. Egg weight (EW), yolk weight (YW), shell weight (SW), albumen weight (AW), yolk percentage, shell percentage, albumen percentage, yolk color, albumen height (AH), specific gravity, and Haugh unit (HU) of eggs from black laying hens fed diets containing marigold extract and paprika at different storage periods.

Effect[2]	EW	YW	SW	AW	Yolk (%)	Shell (%)	Albumen (%)	Yolk color	AH (mm)	Specific gravity	HU
Plant extract	NS	NS	NS	NS	NS	NS	NS	0.012	NS	NS	NS
Storage time	NS	NS	NS	0.0076	NS	NS	0.013	0.005	0.021	0.006	0.0114
Plant extract and storage time interaction	NS	NS	NS	NS	NS	NS	NS	0.019	NS	NS	NS
Storage time	Control[1]										
0	59.84	15.18	5.66	38.99ab	25.46	9.49	65.03a	6.00c	8.55ab	1.086a	93.40ab
7	58.21	14.85	5.77	38.16ab	25.51	9.92	64.55[a]	5.20c	6.77bc	1.074b	80.69 abc
14	55.30	16.91	5.43	32.96b	25.29	9.87	59.52bc	6.28bc	3.65c	1.075b	74.99c
Storage time	Marigold flower extract[1]										
0	59.39	14.63	5.55	39.19ab	24.67	9.35	65.96[a]	9.33[a]	9.51[a]	1.087[a]	96.39a
7	56.35	14.93	5.4	35.98ab	26.49	9.57	63.92ab	8.75abc	5.78c	1.072b	77.27bc
14	55.71	15.64	5.61	34.07ab	28.16	10.8	61.64ab	8.21ab	5.06c	1.075b	74.93c
Storage time	Paprika[1]										
0	59.96	14.44	5.41	40.11a	24.1	9.01	66.87a	10.35a	9.47a	1.084a	93.33a
7	57.66	15.36	5.43	36.79ab	26.82	9.42	63.75ab	9.91abc	6.16c	1.071b	74.49c
14	54.62	15.23	5.11	34.14ab	31.59	9.35	59.04c	8.75ab	5.64c	1.075b	72.64c
CV[3] (%)	7.15	8.16	8.47	9.17	8.42	7.47	3.86	10.05	10.58	6.29	11.48

[1]Means followed by different letters on the same line differ from each other by the Tukey test ($p < 0.05$). [2]NS: not significant. [3]CV = Coefficient of variation.

Regarding egg and shell weight as well as shell percentage there were no significant effects ($p > 0.05$). These results are in agreement with those reported by Galobart et al. (2004), which suggested that natural extracts do not influence animal performance and the quality of the eggs.

The interaction between plant extract and storage time was significant ($p < 0.05$) on egg yolk color. Diets with paprika showed higher egg yolk color, which was 10.35 and 9.33 for paprika and marigold extract, respectively, on day zero of analysis and decreased on the next evaluation days (Table 2).

Moura et al. (2011) demonstrated the ability of pigmentation of these additives while working with the inclusion of natural pigments in sorghum-based diet fed to Japanese quail and observed incremental potentialization of the color evaluated using a colorimetric score. The decrease in color over the evaluated periods can be explained by the process of lipid oxidation, which is a succession of deteriorating reactions that occur during processing, distribution, storage, and final preparation of food containing high amounts of lipid compounds (Soares, 2002). According to Freitas et al. (2011), the pigmentation of the yolk can be altered during storage, obtaining a higher staining in the yolk of eggs submitted to refrigeration.

There was a decrease in albumen height as a function of storage time. These results are similar to those found by Scott and Silversides (2000), and Garcia et al. (2010) who reported that the percentage of albumen decreased with the advance of storage time.

There was no difference ($p > 0.05$) to albumen height, specific gravity, and Haugh unit between treatments with the addition of plant extracts. However, there was a significant effect ($p < 0.05$) of the storage time for these parameters, which indicated that as the storage time increases, the quality of the eggs decrease.

However, even with the effect of storage time, the Haugh Unit score found are within the threshold considered normal for egg quality, which is equal to or above 72 (Musgrove et al., 2004). Bonato et al. (2009) report that the Haugh unit is associated with temperature and storage time rather than the nutrition of laying hens.

The specific gravity decreased as the storage time increased (Table 2), which is directly related to weight loss during storage. Carvalho et al. (2003) and Barbosa, Sakomura, Mendonça, Freitas, and Fernandes (2009) observed similar results on specific gravity values while evaluating eggs of laying hens of different strains and storage time. Bonato et al. (2009) reports that the combination of acidifiers and plant extracts in the diets of laying hens did not alter the specific gravity of the eggs, which is similar to our results.

Conclusion

The extracts of paprika and marigold flower did not influence the quality of eggs of Black Avifran laying hens during the period evaluated.

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