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Effect of *Syzygium cumini* leaves on laying hens performance and egg quality

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ABSTRACT

The aim of this study was to evaluate the effect of *Syzygium cumini* leaves (SCL) in laying hens diets on productive performance, egg quality and lipid oxidation of yolk. A total of 108 Hysex White laying hens were distributed in a completely randomized design with three treatments and six replicates of six birds each. The treatments consisted of SCL inclusion at dietary levels of 0, 5 and 10 g/kg. There was no significant effect of SCL inclusion on feed intake, laying percentage, weight and egg mass, feed conversion ratio, Haugh units, specific gravity, percentage of yolk, albumen and egg shells and shell thickness. However, the inclusion of SCL significantly influenced the yolk color and yolk lipid oxidation measured by TBARS values. Yolk color increased and TBARS values decreased with the inclusion of SCL. The inclusion of SCL in laying hens diets improves pigmentation and lipid stability of yolk.

Key words: antioxidant, egg quality, phytogenic additives, yolk color.

INTRODUCTION

The most frequent deterioration that occurs in foods is lipid oxidation. It affects the quality, flavor, taste and nutritional value and produces toxic compounds. Thus, the use of antioxidants in the feedstuffs or in feed aims to protect or maintain nutritional and energy values of diets (Mariutti and Bragagnolo 2009), being possible to have an extensive effect to the food. For this purpose, synthetic antioxidants commonly used in diets are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). However, studies have shown that these compounds may have some toxicity, resulting in search for natural antioxidants to replace them (Freitas et al. 2013), with focus on phytogenic additives. The phytogenic additives for use in animal feed can be herbs, whole plant or its parts, extracts or essential oils from plants. When added in diets, these additives can provide improvement in feed intake, weight gain, increase digestive secretions and also a protection to damage from oxidative stress due to the antioxidant activity (Ahn et al. 2002).

Studies have been performed to evaluate the inclusion of parts or plant extracts that had already proven *in vitro* antioxidant activity in laying hen diets on performance and improvement in egg quality (Botsoglou et al. 2012, Freitas et al.

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2013), demonstrating the beneficial effects on lipid stability. In this sense, the *Syzygium cumini*, popularly known as "jambolão", "azeitona preta" or "guapê" is a plant belonging to Myrtaceae family plant which different parts are described in the literature by presenting medicinal properties due to different chemical constituents, such as gallic acid, metilgalatic, kaempferol, myricetin, ellagic acid, chlorogenic acid, quercetin and nilocitin, mainly found in the leaves. The *in vitro* antioxidant activity of different extracts of the *Syzygium cumini* leaves has been reported by different researchers (Ruan et al. 2008, Govaris et al. 2010, Ayyanar and Subash-Babu 2012, Chandhary and Mukhopadhyay 2012, Nair et al. 2013, Kaneria and Chanda 2013, Reddy and Jose 2013, Siddiq et al. 2013). Mohamed et al. (2013) reported that the results, especially the antibacterial and antioxidant activity, should encourage investigations of using *Syzygium cumini* leaves as functional food and nutraceutical applications.

Given the above, a preliminary study was conducted to evaluate the effects of adding *Syzygium cumini* leaves (SCL) in laying hens diet on performance and egg quality.

MATERIALS AND METHODS

A trial was conducted in the Poultry Research Center of Animal Science Department at Ceará Federal University - Brazil. A total of 108 laying birds of Hysex White line with 69 weeks of age were housed in galvanized wire cages (25x40x30cm) containing a feeder trough type and a nipple drinker each. The birds used in the assay were selected based on the weight (1.503±0.177 kg) and egg production (84%) to obtain uniform experimental plots, according to Sakomura and Rostagno (2007). The laying hens were distributed in a completely randomized design with three treatments and six replicates of six birds, and the treatments consisted of *Syzygium cumini* leaves (SCL) inclusion at levels of 0, 5 and 10 g/kg in diets. To obtain the SCL, *Syzygium cumini* branches with leaves from different trees were harvested. Then the leaves were detached from the branches and submitted to sun drying. The leaves were spread onto plastic canvas and exposed to the sun for two consecutive days. After drying, the leaves were ground in a 1 mm sieve mill to be included in the rations. The experimental diets (Table I) were formulated to be isoenergetic and isonutritive, considering the nutritional requirements for Hysex White line recommendations and compositional values of feedstuffs proposed by Rostagno et al. (2011). The SCL was added to replace the inert in the rations.

The trial lasted 84 days, divided into four periods of 21 days each. Throughout the experiment, the birds received feed and water *ad libitum* and were subjected to a 16-hour lighting program. The following performance variables were evaluated: feed intake (g/bird.day), egg production per bird per day (%), egg weight (g), egg mass (g/bird.day) and feed conversion per egg mass (kg of diet/kg of eggs). The egg quality was assessed using the variables: Haugh unit, specific gravity, percentage of albumen, yolk and shell, shell thickness and yolk color. The analysis of eggs quality were taken once a week throughout the experimental period. For this, the eggs were collected from each replicate and three of them were randomly selected (avoiding broken, cracked or dirty eggs) to be analyzed.

The specific gravity was determined to obtain the density values of eggs as described by Freitas et al. (2004). The yolk color was performed through the Roche® color fan. To determine the proportions (%) of each constituent of the eggs, egg yolks were separated and weighed on a precision balance (Marte Científica™ Model AD1000, max. 1010g with precision ± 0.01 g). Egg shells were washed and dried at 65 °C for a period of 48 hours and subsequently weighed. Yolk and shell proportions were obtained by the ratio of the weight of each portion and egg weight, while the albumen was

TABLE I
Composition of diets according to SCL inclusion level.

Feedstuffs (g/kg of natural matter)	SCL[1] inclusion level (g/kg)		
	0	5	10
Corn	627.0	627.0	627.0
Soybean meal (45%)	241.2	241.2	241.2
Limestone	88.3	88.3	88.3
Soybean oil	9.3	9.3	9.3
Monodicalcium phosphate	16.6	16.6	16.6
Mineral and vitamin supplement[1]	3.0	3.0	3.0
Salt	3.7	3.7	3.7
DL-Methionine	0.9	0.9	0.9
SCL[2]	0.0	5.0	10.0
Inert[3]	10.0	5.0	0.0
Calculated nutritional and energy composition			
Metabolizable energy (MJ/kg)	11.55	11.55	11.55
Crude protein (g/kg)	161.6	161.6	161.6
Calcium (g/kg)	39.0	39.0	39.0
Avaliable phosphorus (g/kg)	4.0	4.0	4.0
Total phosphorus (g/kg)	6.0	6.0	6.0
Sodium (g/kg)	1.8	1.8	1.8
Total lysine (g/kg)	8.2	8.2	8.2
Total methionine + cystine (g/kg)	6.9	6.9	6.9
Total methionine (g/kg)	3.5	3.5	3.5
Total threonine (g/kg)	6.3	6.3	6.3
Total tryptophan (g/kg)	1.9	1.9	1.9
Linoleic acid (g/kg)	18.7	18.7	18.7

[1] Composition per kg: vitamin A: 2 500 000 IU, vitamin D3: 834 000 IU, vitamin E: 2 000 IU, vitamin K3: 500 mg, vitamin B1: 334 mg, vitamin B2: 1 500 mg, vitamin B6: 334 mg, vitamin B12: 3 333 mg, niacin: 5 000 mg, calcium pantothenate: 2 000 mg, folic acid: 100 mg, biotin: 6.67 mg, choline: 50 g, methionine: 234 g, iron: 6 660 mg, cupper: 2 220 mg, manganese: 20 g, zinc: 17.34 g, iodine: 400 mg, selenium: 100 mg, halquinol: 12 g.
[2] *Syzygium cumini* leaves.
[3] Washed sand.

determined by difference: albumen = 100 - (yolk + shell). To evaluate the shell thickness, pieces were removed from the dried shell with membranes, one from each region of the shell (sharp pole, blunt pole and equatorial region). The thickness of each piece was measured with a micrometer (Mitutoyo™ with measurement of 0.01 mm and accuracy of ± 0.002 mm). The average of the three measures was considered the shell thickness. Lipid oxidation was evaluated by thiobarbituric acid reactive substances (TBARS) in yolk. In the last week of the fourth period, the yolks of three eggs from each replicate were separated from the albumen and placed in a plastic container, homogenized and stored in a freezer (-20 °C) until the analyzes. After 60 days of storage, the yolks were thawed under refrigeration at 4 °C for 24 hours and then transferred to a beaker, homogenized and analyzed. The calibration curve and sample procedures for TBARS were carried out using the aqueous acid extraction method described by Kang et al. (2001). The number of TBARS sample was expressed as mg of malonaldehyde per kg of yolk.

Data were submitted to analysis of variance according to GLM procedure of SAS (2001). The normality and homogeneity of variances were checked for all variables by Shapiro-Wilk and Levene test, respectively, both at 5% of significance and the means were compared using SNK test at 5% of probability.

The experimental procedures followed the protocols approved by the Ethics Committee on Animal Research (CEPA 22/2013) of the Federal University of Ceará.

RESULTS AND DISCUSSION

The feed intake, egg production, egg weight, egg mass and feed conversion per egg mass were not remarkably affected by SCL inclusion at different levels in diets (Table II).

TABLE II
Performance of laying hens fed diet with different levels of *Syzygium cumini* leaves (SCL).

Treatments	Feed intake (g/bird.day)	Egg production (%)	Egg weight (g)	Egg mass (g/bird. day)	Feed conversion per egg mass (kg/kg)
0 g/kg of SCL	107.20	89.23	62.73	55.97	1.92
5 g/kg of SCL	105.29	85.70	63.94	54.43	1.94
10 g/kg of SCL	102.81	85.58	63.24	54.65	1.89
Means	105.10	86.83	63.30	55.02	1.91
ANOVA effects			*p*-value		
Treatment	0.5373	0.5786	0.2294	0.7933	0.7787
CV[1] (%)	6.37	7.77	1.85	7.65	6.36

[1]Coefficient of variation.

Feed intake is an important variable in monitoring commercial laying hens, since the productive performance of laying and egg quality, among other factors, depend on the availability of nutrients for the production processes. In turn, the availability of nutrients is usually ensured by daily feed intake by the birds. Thus, as the feed intake did not differ among treatments, the performance of laying hens fed the inclusion of SCL in the diet was similar to that of birds fed without the addition of SCL. The results obtained for the SCL inclusion on performance are similar to those reported by Botsoglou et al. (2012) whose evaluated olive leaves (*Olea europea* L.) in laying hens diets. These researchers affirmed that the addition of up to 10 g/kg of olive leaves did not interfere in egg production, feed intake and egg weight. However, evaluating the addition of olive leaves in japanese quail (*Coturnix coturnix japonica*) diets, Christaki et al. (2011) found that the addition of up to 20 g/kg increased egg production without major effects on feed intake and egg weight.

Evaluating the egg quality and lipid oxidation of yolk (Table III), there was no significant effect of SCL inclusion on percentage of albumen, yolk and shell, Haugh unit, specific gravity and shell thickness. However, the egg yolk color was increased ($p<0.05$) and a decrease on TBARS values ($p<0.05$) was observed due to the rising levels of SCL. The birds fed diet containing 10 g/kg of SCL produced eggs with higher color value of yolk and lower TBARS value. The increase in yolk color of laying hens fed increasing levels of SCL can be explained by the higher amount of flavonoids in the diet, since these pigments represent the main group of phenolic compounds in SCL (Ruan et al. 2008). According to Garcia et al. (2009), the higher the intake of diets containing carotenoids and flavonoids by birds, the greater the deposition of pigment in the egg yolk and the intensity of the coloration.

In turn, the decrease in TBARS values in the yolk with SCL addition in diet indicates an antioxidant effect preventing peroxidation of yolk lipids. This effect can be associated to an antioxidant activity of different chemical compounds in the leaves of this plant as described by Migliato et al. (2006). Often, it has been reported in *Syzygium cumini* leaves extract the presence of phenolic compounds, besides flavonoids, saponins, steroids, and tannins (Nair et al. 2013) and the highest antioxidant activity of this is directly correlated

TABLE III
Quality characteristics and lipid oxidation of yolk from laying hens fed diets with increasing levels of *Syzygium cumini* leaves (SCL).

Treatments	Albumen (%)	Yolk (%)	Shell (%)	Haugh unit	Specific gravity (g/cm^3)	Shell thickness (mm)	Yolk color[1]	TBARS[2] (mg/kg)
0 g/kg of SCL	65.87	25.91	8.80	79.20	1.078	0.33	6.79c	0.50a
5 g/kg of SCL	65.56	25.79	8.98	76.57	1.078	0.34	7.12b	0.27b
10 g/kg of SCL	65.17	25.94	9.03	76.03	1.075	0.34	7.91a	0.12c
Means	65.53	25.88	8.94	77.27	1.077	0.34	7.27	0.29
ANOVA effects	*p*-value							
Treatment	0.473	0.951	0.505	0.289	0.356	0.341	0.001	0.001
CV[3] (%)	1.49	3.19	3.96	4.63	0.36	4.24	2.04	3.78

Means followed by different letters in the same column are different by SNK test at 5% of probability. [1]Roche® color fan. [2]Thiobarbituric acid reactive substances. [3]Coefficient of variation.

with its phenolic compounds (Reddy and Jose 2013). In addition, Ruan et al. (2008) observed high content of catechin and ferulic acid, affirming that besides phenolic acids, the other complex phenolic compounds in SCL may also be responsible for the antioxidant activity. Nair et al. (2013) affirmed that not only these compounds present antioxidant effect and the high proportion of active compounds can also be due to the presence of other constituents in small amounts or the synergy among them. Moreover, the effects of flavonoids in the cells may be related to the interaction of specific proteins, which are essential for inhibiting a signaling intracellular cascade (Schroeter et al. 2002).

The results obtained with SCL inclusion on egg quality resemble, in part, to those reported in the literature to include plant leaves with proven *in vitro* antioxidant activity. The inclusion of olive leaves (*Olea europea* L.) in japanese quail diets was evaluated by Christaki et al. (2011) observing that the addition of up to 20 g/kg improved the color of the yolk. In turn, Botsoglou et al. (2012) evaluated laying hens producing enriched omega-3 eggs and noted the benefit of adding 10 g/kg of olive leaves (*Olea europea* L.) in diet on lipid stability of yolk, which presented the lowest value for TBARS. In this sense, studies evaluating the *in vitro* antioxidant activity of SCL have demonstrated that, both in DPPH free-radical scavenging activity and ferric reducing power trials, the active components of medium polarity, such as flavonoids quercetin and myricetin, present high correlation with antioxidant action (Ruan et al. 2008, Sultana et al. 2009), and thus resulted in lower TBARS values as observed in this study. According to the results observed by Sultana et al. (2007) when comparing inhibition of linoleic acid oxidation of four different plants, the phenolic compounds present in the SCL had higher antioxidant activity, demonstrating the potential protective effect of flavonoids on polyunsaturated fatty acids, in order to greater participation of these in enriched eggs.

CONCLUSION

The inclusion of *Syzygium cumini* leaves in laying hens diet at levels of up to 10 g/kg improves the color and lipid stability of egg yolk, without harming the performance and quality of albumen and egg shell.

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