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Productive, Qualitative, and Physiological Aspects of Layer Hens Fed with Propolis

Author(s)

Belloni M[I]
Almeida Paz ICL[II]
Nääs IA[II]
Alves MCF[III]
Garcia RG[II]
Caldara FR[II]
Seno LO[II]

[I] MSc in Animal Science, Assistant Professor, UNIGRAM, Dourados, MS, Brazil.
[II] Professor, FCA, Universidade Federal da Grande Dourados. Dourados, MS, Brazil.
[III] MSc in Animal Science, FCA, Universidade Federal da Grande Dourados. Dourados, MS, Brazil.

Mail Address

Corresponding author e-mail address
Rodrigo G. Garcia
FCA, Universidade Federal da Grande Dourados. Dourados, Rod. Dourados - Itahum, Km 12 - Cidade Universitaria, Cx. Postal 533 - CEP 79804-970
67 3410-2001, MS, Brazil.
E-mail:rodrigogarcia@ufgd.edu.br

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ABSTRACT

Laying hens reared under tropical conditions are usually under heat stress. Propolis is known for its pharmaceutical properties, such as increasing cell tolerance to hyperthermia, because of its antioxidants effects. This study aimed at evaluating the influence of different dietary propolis inclusion levels on the performance, egg quality, and bird surface temperature of layers. In this experiment 120 55-wk-old Isa Brown® layers were distributed according to a completely randomized experimental design into four treatments (0, 1, 2 and 3% dietary propolis inclusion levels), with three replicates of ten birds each. Performance and egg quality parameters, and birds' surface temperature were evaluated. Egg production, egg mass, feed intake, and feed conversion ratio were influenced by the treatments. Bird surface temperature was not affected by propolis dietary inclusion. The egg yolk color changed with the treatment ($p<0.05$) when brightness and red and yellow concentration were considered. Evaluators noted a slight difference among treatments during the sensory analysis. The use of propolis in the hens' diet did not improve performance and worsened the eggs' quality.

INTRODUCTION

Acute or chronic stress may significantly affect bird physiology, impairing their live performance and resistance to diseases. Stress factors include climate, environment, nutrition, diseases, as well as management practices, such as cage density and transport (Freeman, 1987).

Live performance and egg quality parameters are commonly used to evaluate the effects of rearing conditions on layers (Alves *et al.*, 2007; Mustaf *et al.*, 2008; Nääs *et al.*, 2010). Worldwide, researchers have studied different types of feed additives to try to mitigate heat stress and to improve hen welfare (Garcia *et al.*, 2002; Galal *et al.*, 2008; Seven, 2008).

Propolis is a complex resinous mixture with dark-yellow to brown color. It is collected by bees from buds, leaves and other parts of trees, such like pines, oaks, eucalyptus, poplars, chestnut trees, *Baccharis dracunculifolia*, *Tabuia* sp., cashew tree (*Anacardium occidentale,* as well from other botanical sources, and mixed with wax. Propolis has anti-microbial, anti-inflammatory and antioxidant pharmacological properties (Nieva Moreno *et al.*, 1999). Propolis, especially its compound CAPE (Caffeic Acid Phenethyl Ester), is used in research on heat stress mitigation, as it improves thermal tolerance (Chen *et al.*, 2009). The antioxidant, cytostatic, antimutagenic, and immunomodulatory properties of propolis are based on its rich flavonoid, phenolic acid, and terpenoid contents (Kimoto *et al.*, 1999; Wang *et al.*, 2003). Current literature indicates that the inclusion of propolis in poultry diets might improve their immune status without influencing their weight gain or performance (Khojasteh & Shivazad, 2006; Seven, 2008).

This study aimed at evaluating the effect of different dietary propolis inclusion levels on the performance, egg quality, and the surface temperature of layers.

METHODOLOGY

The study was carried out at the experimental layer house of the School of Agricultural Sciences, Federal University of Grande Dourados, MS, Brazil (latitude 22 ° 11 'S and longitude 54 ° 56' W), from June 2011 to March 2012. The experimental procedures were approved by the Ethics Committee of the UFGD under protocol number 03/2012.

Birds and husbandry

A total of 120 55-wk-old Isa Brown® layers were randomly distributed into four treatments of 30 hens each. The following treatments were applied: no addition of propolis in the diet (T1, control), dietary addition of 1% propolis (T2), dietary addition of 2% propolis (T3), and dietary addition of 3% propolis (T4). Each treatment included three replicates with ten hens.

Hens were kept in cages in a room with no environmental control, and the management adopted was that recommended by Isa Brown manual (2006). Birds were exposed to a lighting regimen of 17 of (natural and artificial) light in 24 h. Water, and feed were offered *ad libitum.* Eggs were collected once daily.

The treatment diets were manufactured at the university feed mill. The basal diet was a typical layer diet, formulated to meet the nutrient requirements recommended by the National Research Council (NRC, 1994) and contained 2,750 kcal/kg metabolizable energy (ME), 16.50% crude protein, 4% calcium, 0.54% available phosphorus, 2.53% crude fiber, 0.3% methionine, 0.53% sulfur amino acids, and 0.8% lysine. The propolis powder was purchased from a commercial store, and its chemical analysis is shown in Table 1. Propolis was added to the on top of the feed.

Estimated parameters

The following performance parameters were analyzed: feed intake, feed conversion ratio per egg laid, and feed conversion ratio per egg mass. Data were registered and calculated weekly. Feed conversion ratio (FCR) was calculated by dividing the feed intake by the number of eggs produced (EP) or by egg mass (EM).

Table 1 – Chemical analysis of the propolis powder added to the basal diet.

Analysis	Specifications	Results
Mechanical mass	Max. 40% (m/m)	39.80%
Wax	Max. 25% (m/m)	2.59%
Soluble solids	Min. 35 % (m/m)	46.60%
Oxidation activity	Max. 22 s.	16 s
Flavonoid expressed in quercetin	Min. 0,5% (m/m)	3.34%
Minerals	Max. 5% (m/m)	3.77%
Humidity	Max. 8% (m/m)	7.55%

Daily egg production; egg weight; egg specific gravity; eggshell percentage, weight and mineral composition; and yolk color were registered and evaluated daily. Six saline solutions, with densities of 1.060, 1.070, 1.080, 1.090, 1.100 and 1.110, were used to evaluate egg specific gravity at a temperature of 15°C (Castelló *et al.*, 1989). Saline solutions were calibrated using a hydrometer. Egg specific gravity was represented by the solution with the lowest density in which the egg emerged. Eggshell percentage was evaluated after the eggshells were dried at 60ºC for three days, and calculated as the percentage of eggshell weight relative total egg weight (Castelló *et al.* (1989). Egg yolk color parameters L*(luminosity), a* (redness) and b* (yellowness) were determined three different points on the egg yolk surface using a color meter (Minolta® 410R, Konica Minolta, Wayne, U.S.A.). For the sensorial analysis, two eggs in each treatment were randomly selected and cooked for 10 min in boiling water, and were then offered to be appraised by 15 non-trained tasters.

Bird, cage, and roof surface temperature (Ts) were registered weekly, twice a day (07h00min and 13h00min), using a thermal imaging camera (Testo®875, Testo AG, Lenzkirch, Germany). The mean value of surface temperature was obtained by selecting 10 points within the thermal image applying the software (IRTesto® Testo AG, Lenzkirch, Germany), as shown in Figure 1. The adopted emissivity value for the tiles was 0.92 (Nääs *et al.*, 2001). For calculating bird Ts, points were selected in feathered areas (neck and trunk) and featherless areas (comb, wattle and eyes), using the emissivity value of 0.94 for feathered areas and 0.95 for featherless areas, and the camera was placed at 0.72m distant from the birds (Nääs *et al.*, 2010).

(a)
(b)

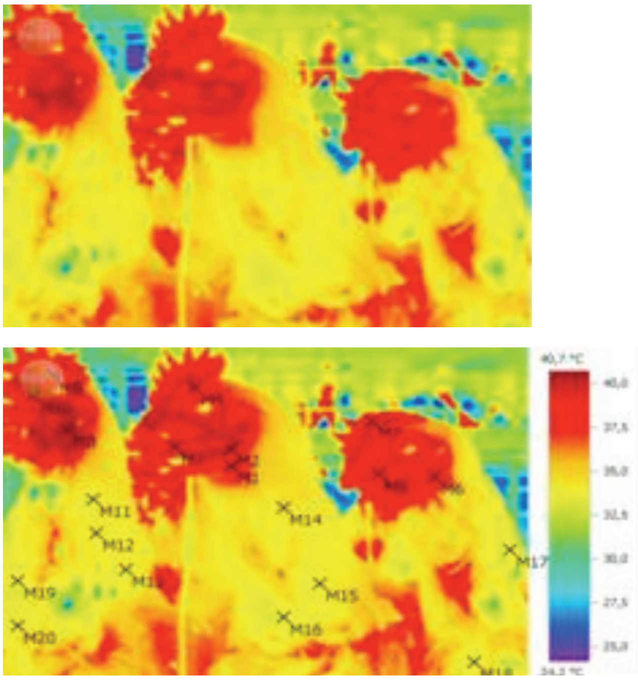

Figure 1. Thermal image of layers (a) and the points marked for calculating mean surface temperature (b).

Ambient temperature and relative humidity were recorded twice weekly at 07h00min and 13h00min, using a digital temperature and relative humidity recorder (Amprobe, Everett, U.S.A.). Luminosity was measured once weekly using a digital lux meter (LX-1010BS, Wensn, ShenZhen, China).

Statistical analyses

All data were subjected to analysis of variance (ANOVA), and the means were compared by the Tukey's test when normally distributed.

For the sensorial analysis, eggs were identified by numbers and the Multiple Comparison test was applied, and the tasters gave scores to the tested samples comparing them to the control sample (ABNT, 1995). Sensorial evaluation data were analyzed using the non-parametric test of Kruskall-Wallis. Data were processed using the software SAS (1996) adopting a probability level of 95%.

RESULTS AND DISCUSSION

Layer performance

Feed intake was influenced by the treatment ($p<0.05$, Table 2): it decreased as dietary propolis inclusion level increased (T3 and T4). This reduction in food intake may be due to the astringent flavor of propolis. Egg production was also affected at the same proportion as feed intake as propolis dietary levels increased. This result differs from that found by Garcia *et al.* (2002) when analyzing the addition of canthaxanthin in layer diets. Galal *et al.* (2008) and Khojasteh & Shivazad (2006) found that high concentrations of propolis in the diet improved performance. However, the levels of propolis added to the layer diets in those studies were lower than those evaluated in the present research.

Table 2 – Egg production (EP), feed conversion ratio per egg mass (FCREM), feed conversion ratio per egg produced (FCREP), and feed intake (FI) of layers fed different propolis levels.

Treatment	EP (%)	FCREM (g)	FCREP (g)	FI/hen (g)
T1	89.9a	2.16±0.2b	94.75±0.0b	121.40±5.4a
T2	85.0ab	2.31±0.2a	102.1±0.0a	124.30±6.6a
T3	82.0ab	2.29±0.3a	102.4±0.0a	114.60±7.7b
T4	79.0b	2.22±0.2b	93.65±0.0b	105.00±5.7b

Results are expressed as mean ± standard deviation. Mean values within a column with no common letter are significantly different ($p<0.05$). T1=control; T2=1% propolis; T3=2% propolis; T4=3% propolis inclusion in the diet

Feed conversion ratio per egg mass (FCREM) was influenced ($p<0.05$) by the treatments, with better results obtained by the hens fed the T1 and T4 diets (Table 2). This result partially agrees with those obtained by Galal *et al.* (2008), who found that the feed conversion ratio of hens improved at high levels of propolis addition to the diet (100-150 mg/kg feed). However, in that study, the worst FCREM result was obtained in the control treatment, differing from the outcome of the present study. The effects of the addition of propolis to layer diets are still controversial. Silici *et al.* (2007) found a significant negative effect of different propolis doses on the weight gain of quails, while Ozkok *et al.* (2013) demonstrated that propolis supplementation in layer diets did not induce any adverse effect on performance, egg quality or survival rate, suggesting that different doses of propolis could be used in egg production.

Egg quality

Egg specific gravity was not affected by the treatments (Table 3, $p>0.05$). The values obtained in the present study are higher than those found by Silva *et al.* (2003), who, however, studied layers in the peak of production, layers were 55 weeks old in the present study. The treatments did not influence eggshell Ca content, which was higher than that found in the literature (Costa *et al.*, 2008) and indicates that eggshell Ca content may not be influenced by the addition of propolis to layer diets.

Table 3 – Egg specific gravity (EG, g/cm³ of egg), eggshell percentage (EP), eggshell calcium (Ca, mg/g) and phosphorus (P, mg/g) contents, and egg weight (EW, g) of layers fed different propolis levels.

Treatment	EG (g/cm³)	EP (%)	Ca (mg/g)	P (mg/g)	EG (g)
T1	1.09±0.0072	9.64	378.25 ±4.72b	1.60±0.01b	66.06 ± 3.31
T2	1.09±0.0072	9.72	385.00±0.82a	1.62±0.01a	65.73 ± 3.75
T3	1.09±0.0084	9.88	377.00±1.83b	1.60±0.01b	64.65 ± 4.32
T4	1.09±0.0085	9.55	375.75±0.96b	1.60±0.01b	65.22 ± 4.08

Results are expressed as mean ± standard deviation. Mean values within a column with no common letter are significantly different ($p<0.05$). T1=control; T2=1% propolis; T3=2% propolis; T4=3% propolis inclusion in the diet

There was no effect of the treatments on egg weight ($p>0.05$), as also found by Ting *et al.* (2011). However, the obtained values are higher than those found by Alves *et al.* (2007). Eggshell Ca and P contents were influenced by the treatments ($p<0.05$), with the hens fed the diet with 1% presenting higher levels compared with those in the other treatments. However, this did not influence egg quality (Couto *et al.*, 2008). Calcium is an important nutrient for birds, and it is an essential for metabolic functions and eggshell formation (Araújo *et al.*, 2008).

Egg yolk color was affected by the treatments (Table 3; $p<0.05$). Luminosity (L*) values were higher in eggs laid by hens fed propolis in their diet compared with the control treatment. On the other hand, redness (a*) value was higher in the yolks of T1 hens (control) relative to the other treatments, while yellowness (b*) values were lower in yolk of eggs laid by hens fed 3% propolis in the diet. The present results differ from those found by Garcia *et al.* (2002) who observed a direct correlation between the increase egg yolk color intensity as the product addition level increased. The effect of propolis in the hens' diet was reduced by the decrease in a* and b* values and by the increase in L* values.

There was no effect of propolis dietary addition ($p>0.05$) on egg weight (Table 4). However, average EW values of the present study were higher than those established by Viana *et al.* (2009), when evaluating the performance of layers fed enzymes to enhance nutrient absorption, which have the same effect attributed to propolis (Seven, 2008).

Sensorial analysis showed that tasters detected a moderate difference between the eggs laid by hens that were fed propolis compared with those laid by the control hens. This result suggests that the addition of propolis to layer feeds may affect consumers' acceptance. Hayat *et al.* (2010) showed that the addition of antioxidants to layer feeds did not increase the acceptance of egg by the tasters, similarly to the findings of the present study.

Rearing ambient and surface temperature

Average ambient temperature (Ta) recorded during the study was 27.9 ± 4.9 ºC, and average relative humidity (RH) was 77%. These rearing conditions may induce heat stress, and are associated with high body surface temperature (Ts) values (Nääs *et al.*, 2010). However, layer performance was not affected ($p>0.05$). Average roof temperature was 56 ºC.

Light intensity was, on average, 91 lx during the experiment, which is within the recommended interval (Freitas *et al.*, 2010), and did influence the studied parameters.

Body Ts values (T1: 32.15 ± 1.96 ºC; T2: 31.79 ± 2.47 ºC; T3: 32.15 ± 2.53 ºC; T4: 31.66 ± 3.01 ºC)

Table 4 – Mean egg weight (g) and yolk luminosity (L*), red (a*) and yellow (b*) values in the eggs laid by layers fed different propolis levels.

	Yolk color			Egg weight
Treatment	L*	a*	b*	
T1	64.45±1.22b	4.22±1.25a	37.06±0.87a	17.21±1.48
T2	65.40±1.47a	2.86±1.16b	36.89±0.83a	16.86±1.54
T3	65.65±1.62a	2.47±1.77b	36.74±0.82ab	17.20±1.54
T4	65.71±1.25a	2.18±1.00b	36.37±0.73b	17.04±1.68

Results are expressed as mean ± standard deviation. Mean values within a column with no common letter are significantly different ($p<0.05$). T1=control; T2=1% propolis; T3=2% propolis; T4=3% propolis inclusion in the diet were not influenced by the addition of propolis to the diet (p>0.05). This may indicate that the addition of propolis did not interfere with the heat exchange of the birds, in agreement with the findings of Viana *et al.* (2009). Mean body Ts varied according to rearing ambient temperature (Table 5) and was not affected by dietary propolis addition. Similar values are reported in the current literature when rearing ambient temperatures are around 40 °C (Mustaf *et al.*, 2008; Nääs *et al.*, 2010).

Table 5 – Mean surface temperature (Ts) of hens fed propolis in feathered areas (neck and trunk), and featherless areas (comb, wattle and eyes), in two periods of the day (morning and afternoon).

Period	Area	Ts (°C)	
		Diet with propolis	Diet without propolis
Morning	Feathered	27.95 ± 3.46	28.54 ± 2.54
Afternoon		28.32 ± 2.68	29.48 ± 2.39
Morning	Featherless	35.77 ± 1.89	35.76 ± 1.38
Afternoon		36.13 ± 1.65	36.28 ± 1.47

Results are expressed as mean ± standard deviation.

CONCLUSIONS

The addition of propolis to the diet did not affect layer performance. However, it influenced egg quality, as determined by egg yolk color differences. Egg flavor was affected by the dietary addition of propolis, which reduced the acceptance of the eggs by tasters during the sensorial analysis. The amount of propolis to be added to layer feeds needs to be further evaluated.

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