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Effect of green propolis on the productivity, nutrient utilisation, and intestinal morphology of Japanese laying quail

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ABSTRACT - In this study, we evaluated the effects of dietary inclusion of ethanolic extract of green propolis (EEGP) on productivity, egg quality, nutrient utilization, and duodenal morphology of 120 Japanese laying quail. The quail were randomly distributed into five treatment groups: one group with no EEGP in the diet (control diet) and four groups with increasing EEGP levels (500, 1000, 1500, and 2000 mg EEGP/kg diet) in the diets. Excreta and diet samples were collected on day 40 and were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash, gross energy (GE), calcium, and phosphorus to evaluate the nutrient and energy use. At the end of the study period, productive performance, egg quality, and duodenum morphology were evaluated. Duodenal sections were obtained from four birds per treatment and were processed for optical microscopy. The EEGP did not affect DM, GE utilization, apparent metabolizable energy, ash retention, feed intake, feed conversion, egg mass, egg quality, crypt depth, or villus height:crypt depth ratio in the duodenum. However, EEGP did increase laying rate, CP, EE, calcium, and phosphorus utilization, as well as villus height and absorption surface area in the duodenum when provided to birds at 1500 and 2000 mg/kg in the diet. Thus, the inclusion of EEGP at 1500 ppm in the diet of Japanese laying quail improves productivity, egg quality, nutrient utilization ability, and duodenal morphology.

Keywords: animal production, egg laying, quail nutrition

Introduction

The use of antibiotic growth promoters in animal feed has been banned by the European Union since 2006 (Tufan and Bolacali, 2017) due to rising consumer demand for sustainable feed-to-food chain and increasing food safety concerns. Therefore, many researchers have started to explore natural alternatives to these antibiotics as feed additives for poultry (Cimrin et al., 2019; Senay et al., 2019), with propolis being one such example.

Green propolis is produced by honeybees from *Baccharis dracunculifolia* DC, commonly known as field rosemary, and contains 3.1-3.3% minerals, 9.8-10.6% crude protein (CP), 45.7-48.7% lipids, and 15.9-20.9% fiber (Machado et al., 2016). It is mainly composed of 3,5-diprenyl-4-hydroxycinnamic acid (Guimarães et al., 2012), which possesses antioxidant (Woźniak et al., 2019), antibacterial (Pereira et al., 2016), antifungal, antiviral (Babińska et al., 2012), anti-inflammatory (Khorasgani et al., 2010), antigenotoxic, and antimutagenic (Roberto et al., 2016) activities.

These properties of propolis could have positive effects on poultry health and performance. According to Hascik et al. (2014; 2015) and Shreif and El-Saadany (2016), the use of propolis improves feed intake, feed conversion rate, and daily weight gain in broilers and laying hens (Belloni et al., 2015; Abdel-Kareem and El-Sheikh, 2017). The use of propolis in the diets of Japanese quail improved feed intake, egg production, egg weight, and feed conversion rate; with this diet, the onset of egg laying started earlier (Mehaisen et al., 2019). Quail chicks under heat stress showed better productive performance when propolis was added to their diets, as reported by Mehaisen et al. (2017).

Related to the egg quality, diets containing propolis may result in better shell weight, Haugh unit (HU), albumen height, yolk index (Abdel-Kareem and El-Sheikh, 2017), egg weight, and shell thickness (Mehaisen et al., 2019) than diets without propolis (control group). Soltani et al. (2019) did not observe any improvement in egg quality when diets of laying hens were supplemented with propolis at 150 or 300 ppm, except for the eggshell thickness, which increased owing to the propolis supplementation.

Seven (2008) observed that digestibility of dry matter (DM), organic matter, CP, and ether extract (EE) were improved with dietary propolis supplementation at 2000 and 5000 mg/kg in laying hens subjected to heat stress. Seven et al. (2011) also found an improvement in the DM and CP digestibility of laying hens under heat stress when fed dietary propolis at 3 ppm. Daneshmand et al. (2012) also demonstrated improved DM digestibility when they added a mixture of propolis, garlic, and mushroom to broiler diet.

Most nutrient transport occurs through the small intestine. Propolis may reduce bacterial translocation and protect the integrity of the intestinal wall (Sabuncuoglu et al., 2007) because of its polyphenolic constituents (Wang et al., 2016). Mahmoud et al. (2017) and Prakatur et al. (2019) demonstrated a beneficial effect of propolis on the intestinal mucosa; they observed increased villus height (VH) and absorption surface area (ASA) in broilers fed propolis.

Studies on effects of ethanolic extract of green propolis (EEGP) in quail production are scarce. Therefore, in this study, we evaluated the effects of EEGP on productive performance, egg quality, nutrient digestibility, and duodenal morphology of Japanese laying quail.

Material and Methods

The research was conducted in Rio Verde, GO, Brazil (17°47'53" S and 50°55'41" W, 715 m), after being approved by the institutional committee on animal use (case number 04/18, approved on May 15, 2018).

A total of 120 Japanese laying quail (45 days old, and initial body weight of 133.7±3.5 g) were used in this study, which lasted for 84 days. Birds were randomly divided into five treatment groups with six birds per group, and there were four replicates per group. All quail in group 1 (control) were fed a basal diet (Table 1), while those in groups 2 to 5 were fed the same basal diet supplemented with 500, 1000, 1500, or 2000 mg EEGP/kg diet, respectively. The EEGP, composed of 30% green propolis resin and 70% cereal alcohol, was purchased from Apiary MelBee, São José dos Campos, SP, Brazil. The EEGP used had a pH of 4.04 and contained 8.8% dry residue, 0.14% ash, 4% lipid, 0.7% nitrogen, and 1.19 mg EQ/g total flavonoids. The dry residue analysis of EEGP was performed according to the method described by Soares et al. (2017), ash and nitrogen were determined according to Silva and Queiroz (2002), lipids according to Bligh and Dyer (1959), and total flavonoids according to Devequi-Nunes et al. (2018). Total flavonoids were expressed in equivalents of quercetin (EQ), and the obtained equation was $y = 0.1402x + 0.1332$, $R^2 = 0.99$.

Birds were housed in galvanized wire cages (25 L × 15 H × 33 W, cm) equipped with gutter-type feeders and drinkers and had free access to feed and water. Birds were maintained under 15-h light for the first seven days, and light duration was gradually increased by 30 min every seven days until it reached 17 h, which was maintained until the end of the study period.

The excreta produced by the 85-90-day-old birds were collected twice a day to evaluate digestibility. The rations were weighed at the beginning and end of the total excreta collection period to calculate average feed intake. Cages were lined with trays coated with properly identified plastics, which were removed and replaced after each collection (12-h interval), and the collected excreta were stored in freezers. Excreta were subsequently thawed, homogenized, weighed, dried in a forced-ventilation oven for 72 h at 55 °C, and ground for further analyses. The excreta and feed samples were analyzed for DM, CP, EE, gross energy (GE), ash, calcium, and phosphorus contents, as described by Silva and Queiroz (2002) to estimate the metabolic coefficients of the nutrients and energy present in the diet. Apparent metabolizable energy corrected for nitrogen balance was also calculated (AMEn) as follows: AMEn (kcal/kg) = $[GE_{ingested} - GE_{excreted} + 8.22 \times NB]/DM$ intake, in which NB is the nitrogen balance (ingested nitrogen − excreted nitrogen).

The evaluated productive parameters were feed intake (g/d), feed conversion (kg/kg eggs and kg/dozen eggs), laying rate (%), and egg mass (g/bird/d). The number and weights of eggs were recorded daily during the study period, and egg mass was calculated by multiplying the number of laid eggs by their weight.

Two hundred normal eggs were randomly selected from the eggs laid during the final three days of the study (five groups × four replicates per group × 10 eggs per replicate) to assess the following qualitative traits: weight (g), specific gravity (g/cm^3), pH, and HU of egg, yolk and albumen weight (g), percentage (%), and index; and eggshell weight (g), percentage (%), and thickness (mm). The specific gravity of eggs was determined by immersing them in saline solutions of different densities (1.05-1.10 g/cm^3). Haugh unit was calculated using the following formula: $HU = 100 \times \log (H - 1.7 \times W^{0.37} + 7.6)$, in which H is albumen height (mm) and W is egg weight (g).

Albumen weight (g) was calculated as the difference between the weight of the entire egg and the combined weight of the yolk and eggshell (g). Percentages of yolk, albumen, and eggshell were determined by the following formula: yolk (%) = [yolk weight (g)/egg weight (g)] × 100. Albumen and eggshell weights were substituted in the formula as necessary. Yolk and albumen indices were determined by dividing their heights (mm) by their respective diameters (mm), with height and diameter being measured using a digital caliper.

After the egg content was removed, eggshells were dried and weighed, and shell thickness (mm) was obtained by calculating the mean of three thickness measurements taken at three different points with a digital caliper (accuracy of 0.01 mm): at the two poles and in the lateral region of the egg.

Twelve hours before the end of the study period, food was removed from all cages and four quail from each treatment were randomly selected and euthanized; sections (5 cm in length) were excised from the duodenal handle region of their duodena. These sections were flushed with saline solution

Table 1 - Composition and chemical analysis of the basal diet offered to laying Japanese quail

Ingredient	g/kg as fed	Calculated analysis[3]	g/kg as fed
Yellow corn	538.1	Crude protein	193.8
Soybean meal	325.0	Calcium	31.7
Soybean oil	32.0	Available phosphorus	3.30
Limestone	74.0	Total lysine	12.9
Dicalcium phosphate	12.0	Total methionine	7.80
L-lysine, 78.8%	2.9	Total methionine + cystine	10.80
DL-methionine, 99%	2.0		kcal/kg
NaCl	4.0	Metabolizable energy	2900
Premix[1]	8.0		
Inert[2]	2.0		
Total	1000		

[1] Enriched with: vitamin A, 10,000 IU; vitamin D3 3000 IU; vitamin E, 25 IU; vitamin K3, 3 mg; vitamin B1, 1.96 mg; vitamin B2, 6.4 mg; vitamin B6, 2.94 mg; vitamin B5, 20 mg; vitamin B3, 35 mg; folic acid, 1.2 mg; biotin, 0.1 mg; choline, 0.363 g; lysine, 0.48 g; methionine, 3 g; phytase, 300 U; Mn, 70 mg; Zn, 60 mg; Fe, 25 mg; Cu, 12 mg; I, 1.23 mg; Se, 0.33 mg; malquinol, 30 mg; antioxidante, 3.6 mg; MOS, 0.12 g.
[2] Ethanolic extract of green propolis was included in the diet at the expense of the inert.
[3] According to Rostagno et al. (2017).

and immediately placed in 10% formalin. After 24 h the sections were transferred to 70% ethanol solution. The samples were then embedded in paraffin and processed for optical microscopy. Villus height (VH, µm), villus weight (VW, µm), and crypt depth (CD, µm) were measured, and the VH:CD ratio was calculated. Absorption surface area (ASA, μm^2) was calculated using the following formula: ASA (μm^2) = VH (µm) × width at 50% VH (µm).

Data were subjected to an analysis of variance followed by SNK tests at a 5% probability level using SISVAR computer package version 5.6 (Ferreira, 2011).

Results

The digestibility coefficients of DM, metabolization coefficient of GE, and AMEn were not affected (P>0.05) by EEGP inclusion; however, the digestibility coefficients of CP (P<0.001) and EE (P<0.005), as well as the retention coefficients of calcium (P<0.008) and phosphorus (P<0.013) were improved in birds fed diet supplemented with 1500 mg/kg propolis (Table 2) compared with birds in the control group and in the group fed diet supplemented with 500 mg/kg EEGP.

The inclusion of 1000, 1500, and 2000 mg/kg EEGP in the diet of Japanese laying quail significantly increased egg-laying rate (P<0.001, Table 3).

Green propolis extract inclusion in the diet did not influence quail egg quality in (P>0.05); however, it did reduce eggshell thickness (P<0.007, Table 4).

Villus height (P<0.001) and ASA (P<0.02) were significantly greater in quail fed 1500 and 2000 mg/kg of EEGP compared with the control group and the group fed the diet containing 500 mg/kg propolis (Table 5).

Table 2 - Nutrient and energy utilization in Japanese quail fed ethanolic extract of green propolis (EEGP)-supplemented diet

EEGP (mg/kg)	DCDM (%)	DCCP (%)	DCEE (%)	MCGE (%)	AMEn (kcal/kg)	AshR (%)	CaR (%)	PR (%)
0	73.31	81.41b	62.92b	82.43	2800	53.22	55.86bc	43.91b
500	73.24	78.43c	57.29b	81.44	2891	51.87	53.05c	41.15b
1000	73.61	83.81ab	79.18ab	80.35	2823	53.28	57.84bc	44.11b
1500	74.98	85.12a	86.51a	83.64	2975	57.50	71.16a	54.67a
2000	73.51	82.36ab	95.96a	80.96	3070	51.87	67.15ab	56.80a
SEM	0.79	0.65	6.01	0.95	138	2.47	3.07	3.03
P-value	0.549	0.003	0.005	0.072	0.266	0.513	0.008	0.013

DCDM - digestibility coefficient of dry matter; DCCP - digestibility coefficient of crude protein; DCEE - digestibility coefficient of ether extract; MCGE - metabolization coefficient of gross energy; AMEn - apparent metabolizable energy corrected for nitrogen balance; AshR - ash retention; CaR - calcium retention; PR - phosphorus retention; SEM - standard error of the mean.
Means in the same row not sharing a common letter are significantly different (P<0.05).

Table 3 - Productive performance of Japanese quail fed ethanolic extract of green propolis (EEGP)-supplemented diet

EEGP (mg/kg)	FI (g/d)	FC (kg/kg)	AC (kg/dozen)	EW (g)	EM (g/d)	LR (%)
0	29.66	2.75	0.403	11.83	10.92	88.29b
500	30.73	2.88	0.420	11.96	10.98	87.80b
1000	31.23	2.75	0.407	11.99	11.15	96.40a
1500	30.59	2.67	0.378	12.10	11.31	94.54a
2000	29.58	2.51	0.372	12.36	11.48	95.33a
SEM	0.86	0.11	0.016	0.29	0.32	1.43
P-value	0.617	0.279	0.249	0.764	0.290	0.001

FI - feed intake; FC - feed conversion; EW - egg weight; EM - egg mass; LR - laying rate; SEM - standard error of the mean.
Means in the same row not sharing a common letter are significantly different (P<0.05).

Table 4 - Egg quality of Japanese quail fed ethanolic extract of green propolis (EEGP)-supplemented diet

EEGP (mg/kg)	EW (g)	SW (g/cm³)	HU	pH	Yolk		Albumen		Eggshell	
					Weight (g)	Index	Weight (g)	Index	Weight (g)	Thickness (mm)
0	12.35	1.070	98.50	7.25	4.35	0.513	7.77	0.166	0.98	0.326a
500	12.10	1.071	96.93	7.35	4.19	0.504	7.51	0.152	0.90	0.291b
1000	11.96	1.072	94.58	7.39	4.13	0.604	8.67	0.147	1.08	0.287b
1500	11.83	1.073	96.52	7.39	3.97	0.507	7.33	0.137	1.01	0.286b
2000	11.99	1.073	95.79	7.52	3.90	0.551	8.36	0.145	0.95	0.289b
SEM	0.29	0.002	1.90	0.07	0.19	0.032	0.44	0.009	0.05	0.005
P-value	0.764	0.846	0.381	0.221	0.519	0.182	0.215	0.295	0.129	0.007

EW - egg weight; SW - specific weight; HU - Haugh unit; SEM - standard error of the mean.
Means in the same row not sharing a common letter are significantly different (P<0.05).

Table 5 - Duodenal morphology of Japanese quail fed ethanolic extract of green propolis (EEGP)-supplemented diet

EEGP (mg/kg)	VH (µm)	CD (µm)	VH/CD	ASA (µm²)
0	697c	94	5.87	84416c
500	761b	103	7.32	81870c
1000	807b	87	6.47	101413bc
1500	869a	92	6.84	111494ab
2000	921a	104	6.76	128191a
SEM	19	6	0.63	5168
P-value	0.001	0.319	0.597	0.002

VH - villus height, CD - crypt depth, VH:CD - villus height:crypt depth ratio; ASA - absorption surface area; SEM - standard error of the mean.
Means in the same row not sharing a common letter are significantly different (P<0.05).

Discussion

The digestibility coefficients of CP and EE, and the retention coefficients of calcium and phosphorus were improved in birds fed diets supplemented with propolis. Propolis contains phenolic acids, mainly represented by benzoic acid and its derivatives (Kurek-Górecka et al., 2014). Benzoic acid increases the digestibility of nutrients, mainly minerals, as shown by Papadomichelakis et al. (2011) with rabbits, and by Diao et al. (2016) with pigs, owing to its antioxidant properties and because it decreases the pH of the digesta and increases the activities of trypsin, lipase, and amylase in the gut (Diao et al., 2016). Propolis also contains enzymes, such as glucosidase (Saeed et al., 2017), α-amylase, β-amylase, α-lactamase, β-lactamase, maltase, esterase, and transhydrogenase, as well as large amounts of essential and aromatic oils (Kurek-Górecka et al., 2014). Because of its antibacterial and antioxidant properties, propolis can improve the mucosa of the small intestine (Abdel-Mohsein et al., 2014; Prakatur et al., 2019; Xue et al., 2019) and, consequently, improve the digestion and absorption of nutrients. Similar results were reported by Seven (2008) and Seven et al. (2011), working with laying hens.

The inclusion of EEGP in the diet of quail did not improve their feed intake, egg weight, feed conversion, and egg mass, similarly to the findings of Tayeb and Sulaiman (2014) with quail and Soltani et al. (2019) with laying hens. However, Belloni et al. (2015) reported that 2 and 3% dietary propolis reduced the feed intake of layers due the astringent flavor of propolis. This effect was not observed in the present study, probably due the lower levels of propolis used.

The inclusion of 1000-2000 mg/kg EEGP in the diet of Japanese laying quail increased their egg-laying rate compared with the control group and quail fed diet supplemented with 500 mg/kg EEGP. Egg production requires large amounts of energy to increase the triglyceride synthesis needed for the formation of the yolk; thus, the improvement in laying rate may be due to the increased utilization of proteins and lipids from the diet caused by EEGP supplementation. This result is corroborated by

Shreif and El-Saadany (2016) and Abdel-Kareem and El Sheikh (2017) and differs from the results obtained by Zeweil et al. (2016) and Soltani et al. (2019), who did not observe any propolis-induced differences in egg production of Japanese laying quail (250 and 500 mg/kg propolis) and laying hens (150 and 300 mg/kg), respectively. However, Belloni et al. (2015) reported a reduction in feed intake and egg laying rate caused by the inclusion of propolis in laying hen diets at 3% of the total diet, which is higher than the levels used in the present study.

In terms of egg quality, EEGP supplementation reduced only eggshell thickness. Mean eggshell thickness ranges from 0.17 to 0.30 mm for quail eggs (Ergun and Yamak, 2017); therefore, the results of this study were within the normal values for quail eggs and did not influence eggshell quality, since specific weight and eggshell weight were not affected. Specific weight is an indirect measure of eggshell quality and is strongly positively correlated with eggshell thickness (Kibala et al., 2018). In agreement with the results of the current study, Belloni et al. (2015), Zeweil et al. (2016), and Soltani et al. (2019) reported that propolis had no effect on egg quality (egg weight, specific weight, shell weight, Haugh unit, and yolk and albumen indices). The mechanism underlying the reduction in eggshell thickness, despite high calcium retention, remains unclear. Recent studies have shown that propolis has estrogenic and anti-osteoclastic activities (Darmadi and Mustamsir, 2016; Zingue et al., 2017; Zhang et al., 2018) and stimulates bone formation (Al-Molla et al., 2014; Okamoto et al., 2015; Pereira et al., 2018). It is possible that the absorbed calcium is directed to the bones of the bird (Bansal et al., 2013) instead of the eggshells, because there are negative correlations between weight and percentage of ash in the tibia and eggshell thickness (Kim et al., 2005) and between bone medullary density and eggshell percentage (Saki et al., 2011) in laying birds.

On the other hand, Ozkok et al. (2013) and Zeweil et al. (2016) showed that propolis had no effect on eggshell thickness, while Seven et al. (2016) and Soltani et al. (2019) reported an improvement in the eggshell thickness caused by propolis supplementation in the diets of Japanese laying quail (4000 mg/kg dietary propolis) and hens (150 mg/kg dietary propolis), respectively.

The inclusion of EEGP improve villus height and ASA in the duodenum. Longer and wider villi are associated with higher intestinal surface area and greater absorptive capacity (Laudadio et al., 2012). The increase in the ASA of the duodenum may have contributed to higher digestibility coefficients for CP and EE, as the duodenum is the main site for the digestion and absorption of fats and proteins (Kiela and Ghishan, 2016).

This result is consistent with that of Belloni et al. (2015), who showed that villus height was greater in the duodena and ilea of laying hens fed diet supplemented with propolis compared with the control group, increasing the area of contact for digesta and mucosa. Chegini et al. (2019) and Prakatur et al. (2019) also reported that propolis supplementation improved villus height in the jejuna and duodena of broilers, respectively.

The results of the present study can be attributed to the beneficial effects of the biologically active components of propolis that participate in controlling pathogenic bacteria and reducing bacterial translocation due to its antioxidant and antimicrobial properties (Abdel-Mohsein et al., 2014; Al-Ani et al., 2018), avoiding damage to the intestinal mucosa, which could lead to reductions in the morphometric variables of the intestinal villi.

Conclusions

The inclusion of ethanolic extract of green propolis at 1500 ppm in the diet of Japanese laying quail is thus recommended, because it improves protein and fat utilization, calcium and phosphorus retention, and egg production, and increases the duodenal absorption surface.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M.C. Oliveira. Funding acquisition: C.A. Pieroni, W.L.R. Santos and M.A.D. Oliveira. Investigation: W.L.R. Santos and M.A.D. Oliveira. Methodology: M.C. Oliveira. Project administration: C.A. Pieroni and M.C. Oliveira. Supervision: M.C. Oliveira. Writing-original draft: M.C. Oliveira and L.B. Mascarenhas.

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