Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v44i1.52845

Replacement of performance enhancers by propolis ethanol extract in broiler diets

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ABSTRACT. The present study was carried out to evaluate the substitution of synthetic performance enhancers by ethanolic propolis extract (EPE) in the diet of broilers and their effects on performance, carcass yield, noble cuts, viscera and bed quality. A completely randomized design was used, with four treatments and six replicates each, totaling 24 experimental plots with 20 birds each. The experimental treatments were: negative control, without the inclusion of performance improvement and EPE-free; positive control, containing avilamycin performance enhancer; Inclusion of 0.1% of EPE and; Inclusion of 0.2% EPE. The analyzed data were submitted to analysis of variance and applied the SNK test at 5% probability. There was no effect of the inclusion of additives on feed intake and feed conversion in the periods from 1 to 7, 1 to 21, and from 1 to 42 days of age. Weight gain was lower in birds receiving feed with the inclusion of 0.1% EPE in the period from 1 to 7 days, not differing from the other treatments in the other evaluation periods. Carcass characteristics, cuts, and viscera and bed moisture were not influenced by the treatments used. The inclusion of ethanol extract from propolis as a performance enhancer up to 42 days of age provided similar results to the use of performance enhancers based on avilamycin, thus being an efficient substitute in poultry production.

Keywords: cutting poultry; growth stimulators; natural extracts; phytotherapy; antibacterial properties.

Received on March 29, 2020.
Accepted on June 2, 2020.

Introduction

Brazilian poultry has shown high growth rates in recent decades and stands out in the agricultural sector among the economic activities that have developed the most due to advances in nutrition, management, sanity, and genetics (Vogado et al., 2016). Research in broiler nutrition has brought great advances, ensuring the supply of animal requirements. Precision nutrition in poultry farming allows the formulation of diets containing the exact nutritional levels of the daily requirement of the animal (Bailey, 2020). Also, several compounds can be added to the diet to increase the feed efficiency of birds (He et al., 2019). As an example of an additive, we can mention antimicrobial performance enhancers, mainly antibiotics and chemotherapy (Zanu et al., 2020), which are products that have proven efficacy on animal productivity and health and are widely used in commercial broiler productions (Ali et al., 2017). The inclusion of this ingredient in diets has as main objective the selection of bacteria beneficial to the intestine, improving the intestinal health of birds, ensuring greater use of the nutrients offered by the diet (Abudabos, Alyemni, Dafalla, & Khan, 2016; Meimandipour, Nouri Emamzadeh, & Soleimani, 2017).

The large-scale use of antibiotic-based performance enhancers in poultry breeding has been questioned and discouraged (Shirzadi et al., 2019). There is a growing concern related to the use of subtherapeutic concentrations of antibiotics in diets and the emergence of resistant pathogenic microorganisms or the presence of residues of these products in an animal carcass, leading consumers to question this use (Biswas, Mohan, Raza, Mir, & Mandal,2019; Tang et al., 2019).

Obtaining a substitute, at the height of these agents, and better understanding the mechanisms of action of antimicrobials as performance enhancers, has been the focus of research by several scholars and the proposals, so far presented, are diverse: prebiotics (Chacher et al., 2017, Biswas et al., 2018), probiotics (Valentim et al., 2018; Ferdous et al., 2019), exogenous enzymes (Cowieson & Kluenter, 2018) and functional plant extracts, originated from plants that have nutraceutical properties as a proposal to remove antimicrobials (Gandra et al., 2015).

Propolis is a resinous, balsamic and gummy substance produced by bees (Casquete, Castro, Jácome, & Teixeira, 2016) originating in the first instance from plant exudations, such as resins and liquids secreted during the initial development of leaf and floral buds, in addition to several other parts of the plant such as shoots, leaves, crevices, and bark of tree trunks (Costa, Barbosa, Holanda, & Flach, 2016) and this substance collected by bees is added wax so that the mixture becomes moldable and during this process, bees add secretions from their glands and pollen (Coelho et al., 2010).

Propolis has numerous active ingredients with biological activity, such as flavonoids, phenolic acids, coumarins, vitamins, and trace elements (Genova, Rodrigues, Martins, Uczay, & Henriques, 2020). It is known primarily for its antimicrobial, antibiotic, antioxidant, immunostimulant, anti-inflammatory, immunomodulatory, hypotensive, healing, anesthetic, anticancer, anti-HIV and anticarcinogenic, antifungal, antiviral, antiprotozoal, anti-antiproriogenic, photoinhibition (Pinto, Prado, & Carvalho, 2011), and may thus be a possible source of substitution for antimicrobial antibiotics used as performance enhancers in broilers.

Because of the above, the objective of this study was to evaluate the replacement of antibiotic-based growth promoter (Avilamycin) with ethanol extract of propolis in diets for broilers in the total production period (1 - 42 days) and to evaluate its effects on performance, carcass yield, noble cuts, edible viscera, and bed moisture.

Material and methods

The experiment was conducted in the experimental shed of Poultry cutting of the Federal Institute of Education, Science, and Technology (IFMG- Bambuí campus). The project was submitted and approved by the animal research ethics committee of the Federal University of Lavras, under protocol number 0033-2013.

A total of 480 single-day-old male broiler chicks of the Cobb 500® strain were used. The chicks were acquired from a commercial hatchery, vaccinated against Marek disease at one day of age and raised in a conventional shed, with northeast-southeast orientation, built-in masonry with wooden structure and roof in ceramic clay tiles. The shed has 48 boxes divided into two sidelines with 24 boxes in each row, with an individual area of 2.6 square meters.

The experimental shed was equipped with an external curtain system on the sides with mechanical drive in turnstiles for partial control of temperature and ventilation, fans and nebulization system for temperature and internal humidity control; heating system in 250 W infra-red power lamps, individually installed in each experimental boxing and central lighting system with automatic firing via a timer.

The experiment was conducted in a completely randomized experimental design with four treatments, six replicates, and 20 birds per repetition, totaling 24 plots and 480 animals. The experimental treatments were: negative control (CN) - without the inclusion of performance enhancers; positive control (CP)- the inclusion of antibiotic-based performance enhancers (avilamycin); Treatment 1 - the inclusion of 0.1% propolis extract in diets for all stages of rearing; Treatment 2 - the inclusion of 0.2% propolis extract in the diets for all stages of rearing. The diet was formulated for the rearing phases: Pre-initial phase, from 1 to 7 days; Initial phase, from 8 to 21 days of age; Growth phase, from 22 to 33 days of age and; final phase, from 34 to 42 days of age.

The isonutritive diets were supplied in the full-fat form and were formulated based on ground corn and soybean meal, according to the requirements of the Brazilian Tables for Poultry and Pigs (Rostagno et al., 2011) for male broilers of regular performance, as well as the composition of the ingredients according to Table 1.

The Ethanolic Extract of Propolis (EPE) used in the inclusion of diets was extracted from green propolis, from apiaries of the Serra da Canastra region in the municipality of Bambuí, Minas Gerais State, Brazil. The composition of the product used follows in Table 2.

The litter used in the rearing of the birds came from a previous batch of chickens reared in IFMG, and the ground rice husk was the material used. The treatment is done in the reused bed of two lots of broiler chicken where it was only performed the burning of feathers by fire broom before being placed in the experimental boxes. The reused bed was chosen to provide greater challenges during the experimental period, being used from the first day of the life of the birds. The daily management consisted of rummaging the bed, cleaning the drinking fountains, supply of feeders, management of curtains, and heaters (infrared lamps), mortality control, height of drinking fountains, and feeders.

Table 1. Composition of basal diets for broilers Coob 500® males of regular performance in the different stages of rearing.

Ingredients	Pre-Initial	Initial	Growth	Final phase
Corn	55.542	61.409	64.029	67.313
Soybeans Bran 45%	37.902	32.704	30.212	27.040
Soybean oil	1.794	1.586	2.074	2.216
Dicalcium phosphate	1.910	1.488	1.244	1.028
limestone	0.914	1.057	0.818	0.831
Salt	0.507	0.482	0.456	0.444
Mineral Premix [1]	0.100	0.100	0.100	0.100
Lysine HCL (99.8%)	0.225	0.184	0.147	0.146
DL-Methionine (99.2%)	0.352	0.277	0.238	0.208
Threonine (98.1%)	0.105	0.064	0.032	0.024
Salinomycin	0.050	0.050	0.050	0.050
Choline Chloride (60%)	0.05	0.05	0.05	0.050
Vitamin Premix[2]	0.050	0.050	0.050	0.050
Additives tested *	0.500	0.500	0.500	0.500
Nutrients	Calculated nutritional composition			
Crude Protein (%)	22.0	20.0	19.0	17.8
Metabolizable Energy (Kcal kg^{-1})	2925	2980	3050	3100
Calcium (%)	0.92	0.86	0.75	0.65
Match Available (%)	0.470	0.384	0.335	0.290
Digestible Lysine. (%)	1.304	1.141	1.045	0.969
Methionine + Digestible Cystine (%)	0.939	0.822	0.763	0.707
Digestible Tryptophan (%)	0.248	0.220	0.207	0.190
Digestible Threonine (%)	0.848	0.742	0.679	0.630
Sodium (%)	0.220	0.210	0.200	0.195

*Space for treatment composition, being: Negative Control: 0.5% kaolin; Positive Control: 0.05% Avilamycin + 0.45% kaolin; Treatment 1: 0.1% EPE + 0.4% kaolin and; Treatment 2: 0.2% EPE + 0.3% kaolin. 1 Warranty Levels (per kg of product): Cobalt (min.) - 200 mg; Copper (min.) – 8,500 mg; Iron (min.) - 50 g; Iodine (min.) – 1,500 mg; Manganese (min.) - 75 g; Zinc (min.) - 70 g. 2 Warranty Levels (per kg of product): Folic Acid– 1,600 mg, Pantothenic Acid– 29,000 mg, Biotin– 60 mg, BHT– 5,000 mg, Niacin– 87000 mg, Vitamin A– 20,000,000 IU, Vitamin B1– 3,000 mg, Vitamin E– 40,500 IU, Vitamin B12– 27,000 mcg, Vitamin B2– 12,000 mg, Vitamin B6– 6,000 mg, Vitamin D3– 5,000,000 IU, Vitamin K3– 4,800 mg.

Table 2. Composition of the Ethanol Extract of Propolis Green.

Analyses performed	Specifications	Result
Wax	1% (m m^{-1})	5.36%
Dry Extract	Minimum 11 % (m v^{-1})	33.37
Oxidation Activity	Maximum 22 seconds	2 seconds
Total Flavonoids (Method Aluminum Chloride)	Minimum de 0.25 % (m m^{-1})	8.32 mg g^{-1} 0.89%
Total Phenolics	Minimum de 0.5 % (m m^{-1})	5.10%
Alcohol Content	Maximum 70° GL	45° GL

The heating system was kept on overnight until the 17th day to maintain the ideal night temperature according to the recommended Cobb 500® (2012) manual. The infrared lamps used in the heating system emitted light to the animals whenever they were connected, providing light to the animals 24 hours a day until the 17th-day. Weighing of birds and feed leftovers was carried out to evaluate feed intake (CR), weight gain (GP), and feed conversion (CA) according to the rearing phases. Food and water were supplied at will throughout the experimental period. Mortality was recorded to correct feed intake of performance data.

At 21 and 42 days of age, bed samples were collected and dry matter analysis was performed to evaluate moisture content. Bed samples were collected at a distance of approximately 1 meter from the water fountains to the center of the boxes (Fogaça et al., 2017). At 42 days of age, two birds were selected with an average weight of each plot, weighed, and identified with adhesive tape with the number of their respective boxing and taken for slaughter after fasting of approximately 8 hours. After transport to the slaughterhouse, they were hung in the noria and desensitized by electronarcosis, and, after desensitization, the sangria did manually with a cut on the jugular. After gentle scalding at a temperature of 52 to 54ºC for 2.5 minutes, the birds were taken to the rotating cylinder with rubber fingers, plucked, and sequentially gutted manually (Valentim et al., 2020).

After evisceration, the carcasses were placed in chillers for pre-cooling, where they left with a temperature of 7ºC. After the chiller, the carcasses were accommodated on a stainless steel mat with holes for 4 minutes to drain the excess water (Valentim et al., 2020). The viscera of each animal were collected, identified, and transported to a room with a bench for analysis. After draining, the carcasses were weighed and carcass yield was calculated with feet, head, and neck and without feet, head, and neck (commercial carcass) using the ratio of carcass weight cooled by live weight after fasting (Valentim et al., 2020).

The yield of noble cuts (breast, thigh, thigh, and wing) was evaluated by the relationship between the weight of the cut and the weight of the cooled carcass without feet, neck, and head (commercial carcass). All cuts were made by a single abattoir employee for standardization and greater uniformity of the parts. From the viscera, the yield of clean, fat-free gizzard and liver, excluding gallbladder, was evaluated about live weight after fasting.

The variables studied were submitted to the statistical premises of normality of residues and the homogeneity of variances and after the analysis of variance was performed through the Sisvar program (Ferreira, 2003) with the application of the SNK test at the level of 5% probability.

Results and discussion

There was no significant difference (p > 0.05) between treatments for CR and AC in the periods from 1 to 7, 1 to 21, and from 01 to 42 days of age (Table 3). Analyzing the Variable GP in the period from 1 to 7 days there was a difference between treatments, and the birds that received 0.1% of EPE presented worse weight gain (p < 0.05) compared to the other treatments.

On the other hand, the GP of the other phases had no effect of the treatments with or without the inclusion of growth-promoting additives as shown in Table 3.

Table 3. Performance of broilers fed from 1 to 7, 1 to 21, and 1 to 42 days with diets supplemented with different levels of Propolis Ethanol Extract (EPE).

Variables	Negative Control	Positive Control	0.1% of EPE	0.2% of EPE	CV[1] (%)	P-value
Phase			1 to 7 days old			
CR (kg)	0.142	0.144	0.142	0.144	4.77	0.1323
GP (kg)2**	0.113a	0.114[a]	0.105b	0.110a	3.55	0.0028
CA (kg kg^{-1})	1.263	1.265	1.356	1.307	5.17	0.2266
Phase			1 to 21 days old			
CR (kg)	1.268	1.309	1.268	1.261	3.79	0.0478
GP (kg)	0.808	0.824	0.811	0.811	3.14	0.0145
CA (kg kg^{-1})	1.570	1.591	1.563	1.557	4.89	0.1176
Phase			1 to 42 days old			
CR (kg)	5.061	5.245	5.165	5.083	2.45	0.3034
GP (kg)	2.909	2.977	2.959	2.935	2.83	0.0789
CA (kg kg^{-1})	1.703	1.725	1.711	1.696	2.73	0.1099

[1]CV(%): Coefficient of variation. 2**Average followed by different letters in the line differs statistically by the SNK test (p < 0.05).

According to these results, one of the possible causes of lower weight gain of birds submitted to treatment with the inclusion of 0.1% EPE about other treatments, including control without the inclusion of propolis and antibiotic, maybe a reduction in the activity of the saccharase enzyme in the pre-initial phase when using EPE. Eyng et al. (2013) observed lower weight gain and feed intake in birds that received different levels of EPE in the period of 1 to 7 days, justifying that supplementation in the pre-initial phase worsened performance due to reduced saccharase enzyme activity. This lower GP in this phase did not impair the development of the lot when analyzed in the other stages of rearing, which, in turn, obtained results similar to the other treatments, having a compensatory weight gain up to 21 days of age.

The lack of effect of the additives added to the feed can be explained by the appropriate experimental environment, the good management conditions, and the nutritional quality of the feed provided, as also reported by Paz et al. (2010) and Araujo et al. (2019). Alani, Alheeti, and Alani (2019), evaluating the use of propolis extract in intercropping or antibiotic substitution in broiler feeding, observed that the inclusion of propolis extract and residue did not interfere with the performance data evaluated. Shaddel-Tili, Eshratkhah, Kouzehgari, and Ghasemi-Sadabadi (2017) studying different levels of properly in the broiler diet, reported that the use of 2,000 ppm of propolis powder in the diet improved body weight gain, feed intake, feed conversion, and production index (p < 0.05), suggesting that this supplementation had a positive effect on animal performance. The reused bed allocated in the experimental plots was not able to provide a greater microbiological challenge in the birds of the research, a fact observed by the result of productive performance of the birds receiving the ration without the addition of propolis or antibiotic being equal to that of birds receiving such food additives in the diet.

There was no significant difference ($p > 0.05$) between treatments for carcass yield variables, noble cuts, liver, and gizzards in broilers at 42 days of age (Table 4).

Table 4. Carcass yield (CR), commercial cuts and edible viscera of broilers fed diets supplemented with different levels of Propolis Ethanol Extract (EPE).

Variables	Negative Control	Positive Control	0.1% of EPE	0.2% of EPE	CV[1] (%)	P-value
R C Cooled (%)	81.38	81.69	82.39	82.30	1.30	0.3196
Commercial RC (%)	71.82	72.19	72.97	72.80	1.58	0.2934
Breast (%)	39.89	40.26	40.80	40.60	3.52	0.7094
Thigh (%)	14.22	14.21	13.54	14.21	3.65	0.0845
Drumstick (%)	15.92	15.99	16.03	16.16	4.54	0.9497
Wing (%)	10.47	10.28	10.07	10.36	3.01	0.1751
Liver (%)	1.74	1.77	1.66	1.71	5.97	0.3185
Gizzard (%)	1.20	1.15	1.11	1.14	9.94	0.6153

[1]CV(%): Coefficient of variation.

Hamed, El-Faham, El-Azeem, and El-Medany (2017) studying the effect of three different levels of propolis and antibiotic (erythromycin) in broiler diets, concluded that birds supplemented with 275 g ton^{-1} of erythromycin or 500 propolis g ton^{-1} would have the same effect on the performance and carcass yield of birds. The effect of different antibiotics, probiotics, and their combinations on performance and carcass yield of broilers were evaluated by Reis and Vieites (2019).

As reported by Kim, Kim, and Kil (2015), the physiological explanation for the replacement of antibiotics by alternative substances such as organic acids may be related to specific antimicrobial activity with additional effects, including pH reduction at digestion, trophic effects on the mucosa of the gastrointestinal tract, and increased pancreatic secretion, causing better performance of birds.

Murakami et al. (2013), evaluating carcass characteristics, gastrointestinal organ weight, intestinal morphometry and digestive enzyme activity in broilers fed different levels of EPE, observed that cutting and carcass yield at 42 days of age were not improved, but supplementation with EPE of 300 g ton^{-1} improved intestinal morphophysiology at 21 days of age. The weight of the organs was not affected.

Gheisari, Shahrvand, and Landy (2017) evaluating the effects of the ethanol extract of propolis as an alternative to antibiotics as a growth promoter in the performance of broilers, indicated that the extract used had no positive influence on the criteria of performance and yield of cuts, but resulted in favorable effects on the biochemical parameters of the blood.

There was no difference ($p < 0.05$) between treatments when analyzing bed quality about moisture content relative to 21 and 42 days of age (Table 5).

Table 5. Percentage of Relative Humidity (RH) of the chicken litter in different stages of rearing broilers fed diets containing different levels of EPE.

Relative Humidity	Negative Control	Positive Control	0.1% of EPE	0.2% of EPE	CV[1] (%)	P-value
UR at 21 days (%)	19.73	20.62	19.70	19.03	8.18	0.9116
UR at 42 days (%)	27.13	27.11	26.54	26.75	6.18	0.8760

[1]CV: Coefficient of variation.

This fact demonstrates that, possibly, there was no higher incidence of intestinal dysbioses between treatments. The growth, cell renewal, repair and defense of the intestinal mucosa are complex processes that involve a multitude of interactions between epithelial cells, interstitial and defense cells present in the lamina propria and those with luminal content (diet, microorganisms, contaminants, toxins) (Macari & Maiorka, 2017). The action of antibiotics and other microbiota modelers has shown improvements in the performance of birds, but the mechanisms involved remain poorly explained (Macari, Mendes, Menten, & Naas, 2014).

According to Ferreira, Oliveira, and Traldi (2004), the bed must have a sufficient humidity rate so that there is no excess dust generation, nor does it retain moisture much, so that it hinders the proliferation of microorganisms that are interesting to their decomposition. Studies report that the chicken litter with 22% moisture is propitiating *Salmonella* spp., *Escherichia coli*, *Listeria*, *Campylobacter*, or *Staphylococcus* spp. Toxigenic (Fogaça et al., 2017). Below these levels already facilitate the incidence of suspended dust. Above 40 to 50% is detrimental to the healthiness of the environment in general (Souza et al., 2019).

Excessive bed moisture, when associated with diarrhea of birds, is often related to the low thickness of the substrate and the shedding of water, creating favorable conditions for ammonia production and promoting the growth of pathogens (Santiago, Passarini, Cerqueira, & da Cunha, 2019). According to Kamimura et al. (2018) beds with humidity above 35% become plastered, causing discomfort to birds, affecting their zootechnical performance, and decreasing resistance to diseases. The bed should be managed so that its humidity is between 20 and 35%, which shows that the variation of moisture found in the present study is not characterized as harmful to the performance of birds.

In the literature (Yadav, Kolluri, Gopi, Karthik, & Singh, 2016; Valentim et al., 2019; El-Hack et al., 2020) the addition of herbal medicines in animal feed can be a tool used to maintain intestinal integrity and thus reduce the number of pathogenic microorganisms that compromise the functionality of the gastrointestinal tract and, consequently, improve performance and animal production. The use of reused chicken litter was not able to provide an efficient sanitary challenging environment for the action of the functional properties of the ethanol extract of propolis.

Many studies (Kavyani, Shahne, PorReza, Haji-abadi, & Landy, 2012; Eyng, Murakami, Pedroso, Duarte, & Picoli, 2017; Valentim et al., 2018) reported that supplementation of broilers with antibiotic substitutes in health-challenge-free environments may not be efficient due to the low incidence of pathogenic microorganisms, causing the active ingredient of the compounds not to have their activity stimulated. Araujo et al. (2019) also reported the use of organic acids and probiotics, isolated or associated, provided inferior performance to those who received antibiotics, not improving the performance and carcass yield of birds under the challenging conditions imposed in the research.

Conclusion

The inclusion of ethanol extract from propolis as a performance enhancer up to 42 days of age provided similar results to the use of performance enhancers based on avilamycin, thus being an efficient substitute in poultry production. New studies including different levels of inclusion of ethanol extract from propolis as well os its action in the intestinal microbiota is a strand to be explored and may thus justify differences in animal performance.

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