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NONRUMINANT NUTRITION

Effect of crude propolis on the performance and feed digestibility of new zealand white rabbits

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ABSTRACT. The objective of this study was to evaluate the performance and digestibility of diets containing different levels of crude propolis for growing rabbits. Forty-eight New Zealand White rabbits, 43 days old, of both genders, were used, distributed in a completely randomized design. There were four diets with different inclusion levels of crude propolis (0.0; 0.5; 1.0 and 1.5%). There was no statistical difference for performance regarding the inclusion levels of crude propolis ($p = 0.85$), as well as for the variables dry matter consumption, neutral detergent fiber and acid detergent fiber ($p \geq 0.07$). Crude protein consumption showed a statistical difference in relation to gender ($p = 0.04$): males showed higher consumption. However, final weight, total weight gain, daily weight gain and feed efficiency were not influenced by the addition of crude propolis ($p \geq 0.37$). Dry matter digestibility, crude protein, neutral detergent fiber and acid detergent fiber were not influenced by the inclusion levels of crude propolis in the diets ($p \geq 0.12$). This study indicates that the inclusion of raw propolis up to 1.5% in the diets does not affect the performance parameters of rabbits.

Keywords: animal feed; natural additive; rabbit breeding.

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Introduction

Rabbits have been part of human food for over two thousand years, and their breeding is a good income alternative for small producers, as they do not need to have much space and capital to start the activity (Frota, 2015). Rabbit breeding is easy to implement, besides being a low-cost technique, and is a source of income for the breeder, not only for meat production, but also for the use of manure, skin, fur, blood and viscera.

The use of dietary additives aims to increase feed efficiency, provide better productive performance and reduce costs in animal production. In this scenario, natural additives have interesting properties for animal feed, and some of them have been studied as a way to replace antibiotics commonly used in animal breeding, such as ionophores. However, there is a need to establish the mechanisms of action of these additives and the real benefits, to enable their use in rabbit breeding (Sakomura, Silva, & Costa, 2014).

Propolis is a name used to describe a complex mixture of resinous substances harvested from buds, flowers and plant exudates by honey bees, to which they add salivary secretions, wax and pollen to yield the final product, which is then brought to the hive, where it performs the protective role (Brasil, 2001).

More than 200 chemical compounds have been identified in propolis, which is mainly known for its antimicrobial, antioxidant, anti-inflammatory activities, among others. In addition, research shows that the use of propolis as a supplement in ruminant and monogastric diets has antibiotic and coccidiostatic properties (Coelho, Silva, & Oliveira, 2010). In this context, it is possible to infer that propolis can act mainly in the control of infectious processes and, consequently, improve the immune response, performance, digestive disorders and feed efficiency of animals.

Therefore, the objective of this study was to determine the best inclusion level of the crude propolis from *Apis mellifera* bees, by evaluating the productive performance and diet digestibility for growing New Zealand White rabbits.

Material and methods

The experiment was carried out in the Experimental Rabbit Breeding Sector of Universidade José do Rosário Vellano (Unifenas), located in Alfenas, state Minas Gerais, from November to December 2017. All procedures performed during the experiment were approved by the Ethics Committee on Animal Use – Ceua, Unifenas, identified by protocol 29A/2017.

Animals and design

Forty-eight growing New Zealand White rabbits from a commercial breeder located in the municipality of Betim, state Minas Gerais, were used. The rabbits were weaned at 35 days of age and started the experimental period at 43 days of age, with an average weight of 1.05±0.04 kg, distributed in a completely randomized design (CRD), in a 2 x 4 factorial scheme, two genders: male and female; and four inclusion levels of crude propolis: 0.0; 0.5; 1.0 and 1.5%, replacing inert kaolin, totaling six females and six males at each inclusion level, with twelve animals per replication.

The experimental diets were formulated to meet the nutritional requirements, according to Blas and Mateos (2010) and were supplied to the animals in pelletized form, as presented in Table 1. The animals were individually housed in galvanized wire cages, where feed and water was offered daily *ad libitum*.

After the adaptation period of three days, in which all animals were fed on the control diet (0.0% propolis inclusion), the animals were weighed and then randomized in the different treatments.

Table 1. Composition of diets containing different inclusion levels of crude propolis for growing New Zealand White rabbits.

Ingredients	Levels of crude propolis (%)			
	0.0	0.5	1.0	1.5
Alfalfa	44.89	44.89	44.89	44.89
Inert	1.70	1.10	0.60	0.10
Dicalcium phosphate	0.28	0.28	0.28	0.28
Propolis	0.00	0.50	1.00	1.50
Lysine	0.06	0.06	0.06	0.06
Methionine	0.15	0.15	0.15	0.15
Maize	12.86	12.86	12.86	12.86
Soybean oil	2.50	2.50	2.50	2.50
Premix[1]	0.40	0.40	0.40	0.40
Salt	0.53	0.53	0.53	0.53
Soybean	7.75	7.75	7.75	7.75
Sorghum	13.96	13.96	13.96	13.96
Wheat	15.02	15.02	15.02	15.02
Total	100.00	100.00	100.00	100.00
Nutrients				
Dry matter, %*	87.60	87.56	87.52	87.48
Crude energy, kcal kg^{-1}**	3965.3	3968.9	3970.64	3972.40
Crude protein, %*	16.77	16.87	16.97	17.07
Crude fiber, %**	13.35	13.36	13.37	13.37
Neutral Detergent Fiber, %*	31.40	31.42	31.44	31.45
Acid Detergent Fiber, %*	20.12	20.13	20.14	20.14
Total lysine, %**	0.87	0.88	0.88	0.88
Total Met + Cis, %**	0.69	0.69	0.69	0.69
Total Ca, %**	0.66	0.67	0.67	0.68
Total P, %**	0.40	0.40	0.41	0.41

[1]Supplied by kg of diet: Zn, 70.00 mg; Fe, 45.00 mg; Cu, 8.00 mg; Mn, 15.00 mg; I, 0.42 mg; Co, 0.10 mg; vit. B_2, 3.60 mg; vit. A, 6,000 UI; vit. D_3, 1,100 UI; vit. B_1, 1.60 mg; niacin, 35.10 mg; folic acid, 1.00 mg; pantothenic acid, 10.00 mg; vit. E, 40 UI; antioxidants, 0.80 mg. Total P: total phosphorus. *Data obtained by laboratory analysis; **Data obtained from food composition tables (Blas & Mateos, 2010).

Inclusion of crude propolis in the diet

The propolis used was acquired from the company 'Entreposto de mel and cera de abelhas', located on Boa Vista farm, in the municipality of Cabo Verde, state Minas Gerais, Brazil. Green propolis was used, which is the most common in the Southern region of Minas Gerais, Brazil (Table 1).

For the inclusion of propolis in the diets, the following procedures were performed: first, three kg of crude propolis were weighed and then frozen for eight hours. After this period, the propolis was ground in an industrial blender for one minute and sieved in a 15 x 32 mm mesh sieve, and incorporated into the feed in quantities related to the treatment levels. Subsequently, the feed was sent to the Nutrimax company, located in the Alfenas (state Minas Gerais, Brazil) Industrial District, where they were pelleted at a thickness of 4 mm and a length of 5 mm by the Chavantes press and pelletizer (model 125 CV).

Experimental period

During the experimental period, performance and digestibility assays were performed, and thermal environment data were collected daily. Thermal environment data were automatically collected, using U12-013 Hobo data loggers, with an accuracy of±0.5°C. These devices recorded air temperature, black globe temperature, relative humidity and dew point temperature at 5 min. intervals. Black globe temperatures were obtained with the aid of an external sensor coupled to the data logger, inserted in a black globe made manually from 150 mm plastic luminaires, painted internally and externally with matt black spray paint. In possession of the data, the Temperature and Humidity Index [THI] (Thom, 1958) were then calculated, in addition to the Globe Temperature and Humidity Index (GTHI; Buffington, 1981).

Every two days, samples were collected from the total diet offered and the leftovers individually, and they were frozen for further analysis.

Performance assay

For the performance assay, the period from 43 to 75 days was considered for the rabbits age. From the final and initial weight, total weight gain (TWG) and daily weight gain (DWG) were determined, as well as feed efficiency (FE), which was calculated by dividing weight gain by feed consumption. From what was supplied and leftovers control, daily dry matter consumption (DMC), daily crude protein consumption (CPC), daily neutral detergent fiber consumption (NDFC) and daily acid detergent fiber consumption (ADFC) were determined over the experimental period.

Digestibility assay

The digestibility assay started at 56 days of age (approximately midway through the experimental period). During this period, the supplied feed, leftovers and feces were collected daily for five consecutive days in each experimental unit. The feces were collected with the aid of a 50% shading screen (sombrite), where it was attached to the bottom of each cage, thus allowing the flow of urine and retention of feces. The total fecal collection was performed in the morning, so that the feces from the same experimental unit were collected in plastic bags, identified, weighed and stored in a freezer at -18°C. After five days, the samples collected from each animal were pooled into a composite sample, identified and frozen for further analysis.

Chemical-bromatological analysis

The chemical-bromatological analyses were performed in the food analysis laboratory of Unifenas College of Agronomy. The samples were pre-dried in a forced circulation oven at 55°C for 72 hours. After pre-drying, the samples were milled in a Willey mill with a 1 mm sieve and stored in plastic containers with lids. Total dry matter (DM) was determined by oven at 105°C for 12 hours, and Crude protein (CP) was analyzed by micro-Kjeldahl steam distillers (Silva, 2012). The fibrous portion was analyzed for fiber content, neutral detergent (NDF) and acid detergent (ADF) with the aid of a Tecnal® apparatus, using the method of Van Soest (1991).

Digestibility calculations

The apparent digestibility (CD) dry matter and crude protein coefficients (CDMS and CDPB, respectively) were calculated using the equation described below (Equation 1):

$$CD(\%) = \frac{(total\ feed\ consumption) - (total\ feces)\ X\ 100}{(Total\ feed\ consumption)}$$

Statistical analysis

All data were subjected to analysis of variance by the SAS statistical software (2016), with gender compared by the F test and propolis inclusion levels assessed by linear and quadratic orthogonal contrasts, considering a significance level of 5%.

Results and discussion

During the experimental period, an average temperature of 23°C and relative humidity of 70% were observed. The thermal comfort zone of rabbits is approximately 15 to 25°C and the relative humidity, 60 to 70% (Mello & Silva, 2012). Thus, it is possible to state that the experimental environment provided to rabbits was within the comfort zone.

The variable final weight showed no statistical difference (p > 0.86) between the levels of propolis increased in the diets. The factor gender did not influence on the final animal weight, but showed a statistical difference when a higher consumption of crude protein was observed, when comparing males and females (p = 0.04; Table 2).

Table 2. Performance of male and female New Zealand rabbits, from 43 to 75 days of age, fed on diets containing different inclusion levels of crude propolis.

Variables	Gender		Levels (%)				p Value			
	F	M	0.00	0.50	1.00	1.50	Gender	Levels Linear	Levels Quad.	Gender* Levels
A	Performance									
Initial weight (kg)	1.05±0.04	1.02±0.04	1.01±0.06	1.04±0.06	1.07±0.06	1.02±0.06	-	-	-	-
Final weight (kg)	2.02±0.04	2.08±0.04	2.03±0.06	2.02±0.06	1.97±0.06	2.19±0.06	0.37	0.12	0.07	0.86
B	Consumption									
DDMC (g)	106.09±1.30	109.13±1.30	108.99±1.85	105.78±1.84	107.14±1.85	108.51±1.84	0.11	0.99	0.22	0.80
DCPC (g)	20.37±0.26	21.05±0.26	20.73±0.37	20.18±0.37	20.72±0.37	21.21±0.37	0.04*	0.24	0.18	0.73
DNDFC (g)	43.24±0.95	45.51±0.95	45.17±0.13	43.21±0.13	43.97±0.13	45.14±0.13	0.10	0.91	0.25	0.81
DADFC (g)	20.93±0.50	22.12±0.48	21.71±0.72	21.25±0.69	21.42±0.69	21.71±0.69	0.10	0.96	0.60	0.79
C	Weight gain									
TWG (kg)	0.978±0.03	1.034±0.03	0.983±0.04	0.973±0.04	0.923±0.04	1.145±0.04	0.37	0.12	0.07	0.86
DWG (g)	31.55±1.38	33.34±1.38	31.71±1.96	31.37±1.96	29.79±1.97	36.93±1.96	0.37	0.12	0.07	0.86
FE	3.58±0.15	3.46±0.15	3.56±0.22	3.64±0.21	3.78±0.21	3.11±0.21	0.76	0.27	0.10	0.91

DMC: Daily dry matter consumption; CPC: Daily crude protein consumption; NDFC: Daily neutral detergent fiber consumption; ADFC: Daily acid detergent fiber consumption; TWG: total weight gain; DWG: daily weight gain; FE: feed conversion; average standard error; F: females; M: males; Levels: inclusion of propolis at 0.0%; inclusion of propolis at 0.05; inclusion of propolis at 1.00% and inclusion of propolis at 1.50%; Treat: treatment; *significant at 5%.

Retore, Scapinello, and Moreira (2012) evaluated the gender factor, and observed that male animals normally have higher nutritional requirements than females and, consequently, this higher requirement of males may result in higher feed consumption and greater weight gain, which contrasts with the results found in this study, where gender influenced crude protein consumption, but with no differences in final weight gain. This result can be explained due to the higher requirement of male animals, which is related to body composition, since males have more muscle and this fact implies higher caloric expenditure with muscle protein turnover, which normally leads to higher maintenance requirements for males compared to females (Bellaver, Monteiro, & Zanatto, 2010). In addition to this aspect, males frequently have larger organ sizes such as liver, kidneys and heart, also leading to higher maintenance requirements when compared to females (Machado, 2012).

Nesi and Demeda (2015) evaluated the performance of animals fed on high protein diets, and found higher final weight and better performance of animals. Klinger, Toledo, and Eggersi (2014) also observed higher final live weight in New Zealand White rabbits of both genders, when fed on a diet with 50% replacement of soybean hulls with alfalfa hay, which resulted in higher protein diets. These results differ from those found in this study, where the inclusion of crude propolis did not affect the performance and final weight of the animals.

The final weight and consumption variables (DMC, CPC, NDFC and ADFC) evaluated in the performance test did not differ (p ⩾ 0.73) with the increase in crude propolis levels in the diet (Table 2B). In this context, it is possible to observe that the inclusion of propolis did not affect consumption or performance parameters, which can be explained by the low inclusion level of propolis (maximum inclusion level of 1.5% in the whole diet), showing that higher levels should be evaluated. These results were similar to those observed by Lui, Oliveira, Caires, and Cancherini (2005), who used New Zealand White rabbits of both genders, and observed that performance parameters were similar for feed consumption, weight gain and final weight, with increasing inclusion levels of probiotics (0.15% *Bacillus subtillis* at a concentration of 109 CFU g^{-1}).

Daily feed consumption (DMC, CPC, NDFC and ADFC) was similar among the different inclusion levels of crude propolis in this study, and may be the main factor not to detect different weight gain values. Consumption is the main driver of performance variables, especially when animals are reared under similar conditions. In this study, the average daily feed consumption among all crude propolis inclusion levels was 108 g over the experimental period. These data are in agreement with studies by Scapinello, Antunes, and Furnan (2003), who evaluated growing New Zealand White rabbits and observed an average feed consumption of 120 g for each animal.

The inclusion level of crude propolis did not increase (p ⩾ 0.37) the weight gain of growing rabbits. Similar results were found in a study by Coloni (2007), who concluded that there was no difference between treatments on final weight gain and total weight gain of New Zealand White rabbits using the ethanolic propolis extract.

There was no difference (p ⩾ 0.76) when evaluating the variable feed efficiency (FE) between the treatments with the different inclusion levels of crude propolis (Table 2C), a result that is in agreement with that found by Garcia (2004), who observed that feed efficiency did not differ between alcohol-containing treatments and different levels of propolis alcohol extract from Norfolk-200 young rabbits (*Botucatu* strain). According to the authors, the inclusion levels of propolis alcohol extract may have affected feed consumption and, therefore, may have interfered with FE.

There was no difference regarding digestibility parameters (p ⩾ 0.29, Table 3). Different results were found by Zanato (2008), in a study in which it was observed that the use of prebiotics in the diets of growing rabbits showed a higher digestibility of DM and CP. According to Prado (2011), the use of crude propolis may affect diet digestibility due to the presence of wax (which is indigestible); thus, the authors indicate that, for the use of propolis, it is necessary to make extracts in order to enable the active substances to be available and remove the waxes. In this study, not removing the wax did not affect diet digestibility, and up to 1.5% of crude propolis could be included in the diet.

Table 3. Digestibility of diets containing different levels of crude propolis and water consumption of New Zealand White rabbits of both genders, from 43 to 75 days of age.

Variables (%)	Gender		Levels (%)				P Value			
	F	M	0.00	0.50	1.00	1.50	Gender	Levels Linear	Levels Quad.	Gender* Levels
Dig. DM	114.01±1.76	116.57±1.69	116.34±2.38	114.93±2.61	113.92±2.29	115.98±2.49	0.30	0.85	0.49	0.59
Dig. CP	21.60±0.37	22.49±0.36	22.15±0.51	21.90±0.56	21.97±0.49	22.15±0.51	0.29	0.91	0.53	0.58
Dig. NDF	47.25±1.23	39.10±1.18	48.70±1.66	48.70±1.82	47.18±1.59	48.72±1.74	0.35	0.56	0.54	0.92
Dig. ADF	23.04±0.63	23.90±0.59	23.72±0.87	23.60±0.91	23.02±0.80	23.50±0.87	0.33	0.77	0.72	0.57

Dig. DM: Dry matter digestibility; Dig. CP: Crude protein digestibility, Dig. NDF: Neutral detergent fiber digestibility, Dig, ADF: Acid detergent fiber digestibility. F: females; M: males; Levels: 0.0% propolis inclusion, 0.05% propolis inclusion, 1.00% propolis inclusion and 1.50% propolis inclusion; Treat: treatment.

Conclusion

The inclusion of crude propolis up to 1.5% does not affect the performance or diet digestibility of New Zealand White rabbits. Further studies are necessary in rabbit breeding in order to provide alternatives for producers with a view to improving production costs and dissemination of rabbit production.

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