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Green propolis extract as additive in the diet for lambs in feedlot

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ABSTRACT - The objective of this paper is to assess the effects of the inclusion of different levels of green propolis extract in the diet of lambs in feedlot on ingestive behavior, nutrients digestibility, physiological parameters and performance. Eight lambs were distributed in double Latin Square with four treatments, corresponding to the inclusion levels (4, 8, 12, 16 mL) of green propolis ethanolic extract (30 g of ground crude propolis was infused in a 100-mL hydroalcoholic solution, 700 mL/L). The diets were composed of *Brachiaria brizantha* cv. MG5 hay and a commercial concentrate (roughage:concentrate ratio was 50:50) in a dry matter basis. No effect was observed on dry matter (31.2 g/kg of BW), crude protein, ether extract, neutral detergent fiber, non-fibrous carbohydrates and total digestible nutrients content (TDN) intakes. No significant effect was seen on the digestibility coefficients, presenting an average of 65.94% of TDN. The green propolis extract levels do not have a significant effect on behavior or physiologic parameters. Seeking to maximize feeding efficiency, the inclusion of 7.60 mL/day (2.1189 mg of dry matter and 0.1123 mg of flavonoids) of green propolis extract in the diet of lambs in feedlot is recommended.

Key Words: additive, *Apis mellifera*, flavonoids, ionophore

Introduction

The animal's response to a certain feed is influenced by complex interactions between diet composition and processing, and consequently the nutritional value, which is defined by three components: dry matter intake, digestibility and energetic efficiency (Van Soest, 1994). Therefore, additives must be evaluated on these variables, with the objective of characterizing the potentiality of use in animal nutrition and feeding.

Propolis is the product of resinous, gummy and balsamic substances that are collected by bees from buds, flowers and plant exudates, and mixed with their salivary secretions, wax and pollen. This serves to seal and protect the honeycomb against insect and microorganism attack as well as to maintain internal temperature and humidity (Brasil, 2001).

The chemical composition of propolis is complex and variable because it is intrinsically related to the floristic and ecological composition of the environment visited by the bees (Ghisalberti, 1979). The combination of these factors affects the pharmacological properties of propolis.

According to Mirzoeva et al. (1997), propolis has bacteriostatic activity against *some gram*-positive and *gram*-negative bacteria, possibly because of changes in the bioenergetic status of the bacterial membrane, which inhibits bacterial motility. The inhibitory propolis action, *in vitro* and *in vivo*, on the deamination of amino acids was reported by Stradiotti Junior et al. (2004), which can mean greater ruminal protein escape, with consequent improvement of production efficiency of ruminants.

In dairy goats, Lana et al. (2005) did not find the influence of propolis extract in feed intake, rumen fermentation, digestibility or milk production and composition. Lana et al. (2007) also found no effect of propolis extract in the diet of milk production parameters of goats in lactation.

Considering the abovementioned facts, this study evaluated the effects of different levels of the dietary addition of green propolis on the behavior, nutrient digestibility, physiological parameters and production performance of lambs confined in metabolism cages.

Material and Methods

The experiment was carried out at the Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil. Eight six-month, crossbred (Suffolk × no breed defined), not castrated male lambs weighing 22.8 kg at the beginning of the experiment and 34.5 kg, at the end of the experiment were used. Throughout the experiment, lambs received

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anthelmintic treatment according to regular FEC analyses (fecal egg counts per gram).

The lambs were housed in metabolism cages for digestibility studies with feeder and waterer. Water and mineral salt were offered *ad libitum*. Braquiaria-grass hay (*Brachiaria brizantha* cv. MG5) from seed harvest, chopped to 5 mm length, was used as roughage. For concentrate, soybean meal, corn meal and minerals were used, with a roughage:concentrate ratio of 50:50 on a dry matter basis (Table 1). Lambs were fed daily at 8 a.m. Diet was offered at ease, with minimum supply of 10% of leftovers.

Crude propolis was obtained from the beekeeping company in Contagem, MG, Brazil, with specification: green propolis derived from "alecrim-do-campo" *(Baccharis dracunculifolia)*, with oxidation level similar or lower than 10 g/kg, was collected once a week in shaded areas with special strips for propolis collection. In order to obtain propolis extracts, 30 g of ground crude propolis were infused in a 100-mL hydroalcoholic solution (700 mL/L). After 10-day infusion, the extract was filtered in paper filter and used as stock solution. The extracts were freshly diluted in deionized water (1:1) before being added to the diets, according to Stradiotti Jr. et al. (2004). Dry matter (m/m) was 278.8 g/kg, flavonoid content (m/m) was 14.9 g/kg and total phenol was 14.7 g/kg in green propolis, analyzed according to Funari & Ferro (2006).

The lambs underwent four levels of ethanolic extract of green propolis in the diet, on a daily administration: 4 mL (dry matter 1.1152 mg and flavonoid 0.0596 mg), 8 mL (dry matter 2.2304 mg and flavonoid 0.1192 mg), 12 mL (dry matter 3.3456 mg and flavonoid 0.1788 mg) and 16 mL (dry matter 4.4608 mg and flavonoid 0.2384 mg).

The experiment consisted of four experimental periods of 21 days, 14 days for adjustment to diet and seven days of sample collection, totaling 84 days of feedlot. In addition, the animals wore bags adapted to feces collection for three days during the period of adaptation to diet. Feed and orts have been quantified and sampled daily. Total collection of feces was conducted with the use of bags adapted to feces collection, every 24 hours for a period of seven days. Feces were collected manually before feeding of animals, then sampled and homogenized, with 10% of the total excreted to formation of composite sample. The diet offered, orts and feces, collected by composite sampling, were quantified. Samples were stored in a freezer (-20°C) for subsequent laboratory analysis. The animals were weighed initially and at every 21 days, after fasting of solids for 16 hours for evaluation of DM intake (DMI) in the percentage of body weight (g/kg BW) and in g/kg of metabolic weight (g/kg $BW^{-0.75}$), daily weight gain and feed efficiency (total weight gain/DMI).

Samples were dried in a forced ventilation oven at 55ºC for 72 h and ground to pass through a 1 mm mesh screen. Diets, orts and feces were analyzed according to Silva & Queiroz (2002) as for dry matter (DM); organic matter (OM); crude protein level from total nitrogen (CP); ether extract (EE) and neutral detergent fiber (NDF). Total carbohydrates (TCHO) were determined using the equation: 100 – (crude protein + ether extract + ash). To determine non-fibrous carbohydrates (NFC), NDF was subtracted from TCHO (Sniffen et al., 1992). Total digestible nutrients (TDN) were estimated according to Sniffen et al. (1992), using the equation: TDN = (CP intake × CP digestibility) + (NDF intake × NDF digestibility) + (CNF intake × CNF digestibility) + (2.25 × EE intake × EE digestibility).

Ingestive behavior of lambs was evaluated at every 21 days for a total of four observations. Each observation started at 8 a.m., at the first daily feeding, and continued for 24 hours. Thus, 96 h of behavioral data were recorded for each lamb. The collection of quantitative data on basic behavioral patterns was based on instantaneous scanning and continuous sampling, according to Altmann (1974) and Martin & Bateson (1993). After, 1-min scans were performed at 10-min intervals over the 24-hour observation period. A chronological framework was used to record the time the lambs spent feeding, ruminating, resting, moving and drinking water.

Table 1 - Chemical composition (g/kg, on dry matter, DM basis) of the total mixed ration

Chemical composition	Ground braquiaria grass	Concentrate[1]	Total mixed ration
Dry matter (g/kg)	905.2	865.5	885.4
Organic matter (g/kg DM)	970.1	938.9	954.5
Crude protein (g/kg DM)[2]	24.8	259.3	142.1
Ether extract (g/kg DM)	11.6	33.5	22.6
Neutral detergent fiber (g/kg DM)	854.6	230.4	54.25
Non-fibrous carbohydrates (g/kg DM)[3]	79.1	415.6	247.4

[1] Ingredients (517g/kg corn meal; 472 g/kg soybean meal; 1g/kg premix mineral).
[2] Crude protein = Ntotal × 6.25.
[3] Non-fibrous carbohydrates = 100 – (crude protein + ether extract + a neutral detergent fiber + ash).

The physiological parameters measured were: breathing rate (BR), by auscultation with stethoscope in the laringo-tracheal region and expressed as breaths per minute, with the average of three measurements; heart rate (HR) obtained with the stethoscope, placed directly in the left thoracic region at the aortic arch height and expressed in beats per minute (Baccari Junior, 1990), with average of three measurements; rectal temperature (RT) determined by introducing a veterinary clinical thermometer, with scale up to 44 ºC, directly in the rectum of the animal, for a minute. Data collection occurred at every 21 days, 7 a.m. and 3:30 p.m.

The animals were distributed in two Latin Squares (4 × 4), with four treatments (inclusion levels of propolis) and eight replications. A basic ethogram was used to quantify lamb behavior. Ingestive behavior, nutrient digestibility, physiological parameters and performance were evaluated using variance analyses and regression. Means were compared using the Tukey test at a significance level of P = 0.05.

Results and Discussion

No effect (P>0.05) of inclusion level of green propolis extract on the nutrients' intake was detected (Table 2). Dry matter intake was 31.2 g/kg of body weight (BW), less than the 36.1 g/kg BW found by Ítavo et al. (2011), in lambs receiving 15 mL of green propolis extract in the diet, with Tifton 85-grass hay (591.6 g NDF/kg DM). It may have been caused by the low quality of roughage of this experiment (854.6 g NDF/kg DM, Table 1), with possible disaccelerated feed passage through the gastrointestinal tract, resulting in lower DMI, possibly by the physical effect. DMI was similar to that recommended by the NRC (1985), which estimates a minimal value of 1000 g DM/day for animals with more than 20 kg of body weight. It is worth stressing that ground hay did not cause digestive or metabolic disorders.

The results of this research corroborate reports from Lana et al. (2007), who found no effect of level of green propolis (0, 1.0, 2.0, 4.0, 8.0 and 12 mL/animal/day) added to the basal diet of goats on the DM, OM, CP, NDF and NFC intake. There was no difference between the inclusion levels of propolis on water intake (Table 2), with average consumption equal to 5.4 L/kg DM. There was no effect (P>0.05) of propolis on the apparent digestibility coefficients of nutrients studied (Table 3).

Apparent digestibility of dry matter (DMD), 0.6204 was lower than 0.6914 obtained by Bolzan et al. (2007), using the 50:50, roughage:concentrate, *B. brizantha* hay and concentrate with corn grain. Ítavo et al. (2009) assessed diets containing corn and sorghum high moisture silages and verified DM digestibility equal to 0.5632, lower than that obtained in this experiment. It can be seen, for NDF digestibility (0.5210), that the use of fibrous fraction of roughage was identical in the different levels of inclusion of propolis extract, which reflected in making maximum use of NFC (NFC digestibility equal to 0.9049), also similar among the different diets. Possibly, absence of effects in DMI, a characteristic of ionophore, is linked to the fact that the voluntary intake has been limited by the physical effect of rumen filling, associated with the low quality hay used (Table 1).

There was no effect of green propolis extract (P>0.05) for the behavior variables evaluated, probably because animals confined present tendency to exhibit similar behavior, due to their being held in individual stalls and having the same diet (Table 4).

Table 2 - Mean values of nutrients and water intake in different levels of green propolis in experimental diets

Item	Green propolis extract level[1] (mL/day)				CV (%)	Mean
	4	8	12	16		
Dry matter (g/day)	873.21	822.54	837.73	827.79	15.34	840.32
Dry matter (g/kg BW)	32.0	31.2	31.1	3.06	15.14	31.2
Dry matter (g/kg $BW^{0.75}$)	73.09	70.59	70.65	69.64	15.15	70.99
Organic matter (g/day)	831.85	783.54	798.19	788.36	15.38	800.49
Crude protein (g/day)	134.84	127.41	129.44	128.46	13.39	130.04
Ether extract (g/day)	20.85	19.47	19.66	19.75	13.59	19.93
Neutral detergent fiber (g/day)	440.95	415.21	423.79	416.36	17.40	424.08
Neutral detergent fiber (g/kg BW)	16.1	15.7	15.7	15.4	17.23	15.7
Neutral detergent fiber (g/kg $BW^{0.75}$)	36.85	35.64	35.73	35.03	15.15	35.81
Non-fibrous carbohydrates (g/day)	235.21	221.47	225.30	223.80	13.46	226.45
Total digestible nutrients (g/day)	577.54	537.18	552.62	542.42	14.88	552.44
Water (L/day)	4.29	4.51	4.41	4.52	18.35	4.43
Water (g/kg BW)	161.0	171.8	170.3	172.4	215.0	168.6
Water (mL/kg $BW^{0.75}$)	365.28	388.44	383.31	389.25	19.46	381.57
Water (L/kg DM)	5.22	5.57	5.62	5.76	26.22	5.54

[1] Means followed by different letters in a same row are statistically different (Tukey test, P<0.05). CV = coefficient of variation.

The average time spent by animals with rumination total 0.3394, similar to the 0.3339% observed by Ítavo et al. (2011), probably due to the same 50:50 ratio roughage:concentrate utilized. However, the time dedicated to the feed intake (0.1498) was shorter in in comparison to the 0.1723 observed by Ítavo et al. (2011) with two daily feedings, which may be related to the positive influence caused by the partition of the diet into two or more daily treatments, with consequent increase in nutrients intake.

There was no effect on the level of inclusion of propolis extract (P>0.05) on the physiological parameters evaluated (Table 5), breathing rate, heart rate and rectal temperature.

The average value found for breathing rate (82.21 breaths/min) was similar to that of 76.3 ± 6.3 breaths/min pointed by Marai et al. (2007) in Suffolk sheep. Average heart rate was 103.62 beats/min, close to the 88.0 ± 11.0 beats/min pointed by Marai et al. (2007). However, Santos et al. (2006) found values between 122 and 141 beats/min, greater than those obtained in this study. Silva & Gondim (1971) claimed that heart rate is a variable subject to a large number of factors, besides temperature, such as age, individuality, temperament and the degree of excitement of the animal. Possibly, at the animal containment for auscultation, there was slight increase in heart rate, without characterization of thermal stress condition.

Rectal temperature average (39.52°C) was within the range considered normal (38.5 – 40.5°C), and the rectal temperature of sheep is equal to 39.1°C (Swenson & Reece, 2006), evidencing that the animals were kept in a suitable environment. The value found for rectal temperature for Marai et al. (2007) for Suffolk sheep created in tropical environments was close to 39.9 ± 0.1°C, whereas 39.72°C was obtained by Santos et al. (2006).

There was quadratic effect (Table 6) of green propolis extract in the diet on average daily gain (ADG) and feed efficiency (FE) of lambs.

Despite the similarity between intake and digestibility on the different levels of propolis inclusion in the diet (Tables 2 and 3), there was no difference in the productive performance of animals, which may be related to differences

Table 3 - Mean values of digestibility coefficients and total digestible nutrients in different levels of green propolis in experimental diets

	Green propolis extract level[1] (mL/day)					
Item	4	8	12	16	CV (%)	Mean
Dry matter	0.6299	0.6156	0.6244	0.6117	4.75	0.6204
Organic matter	0.6754	0.6690	0.6730	0.6701	3.31	0.6719
Crude protein	0.7515	0.7417	0.7564	0.7496	2.68	0.7498
Ether extract	0.6806	0.6313	0.6562	0.6297	14.03	0.6495
Neutral detergent fiber	0.5276	0.5148	0.5224	0.5190	7.10	0.5210
Non-fibrous carbohydrates	0.9015	0.9171	0.9043	0.8967	3.14	0.9049
Total digestible nutrients (g/kg DM)	663.8	656.2	660.5	656.9	3.14	659.4

[1] Means followed by different letters in a same row are statistically different (Tukey test, P<0.05). CV = coefficient of variation.

Table 4 - Mean values of behavior activity of lambs in different levels of green propolis extract in experimental diets

	Green propolis extract level[1] (mL/day)					
Item	4	8	12	16	CV (%)	Mean
Feeding	0.1589	0.1467	0.1441	0.1493	30.45	0.1498
Drinking water	0.0104	0.0069	0.0052	0.0052	127.48	0.0069
Moving	0.0859	0.0903	0.0894	0.0868	38.46	0.0881
Ruminating	0.3481	0.3273	0.3455	0.3368	15.00	0.3394
Resting	0.3967	0.4288	0.4158	0.4219	16.91	0.4158

[1] Means followed by different letters in a same row are statistically different (Tukey test, P<0.05). CV = coefficient of variation.

Table 5 - Daily average breathing rate, heart rate and rectal temperature of lambs in different levels of green propolis extract in experimental diets

	Green propolis extract level[1] (mL/day)					
Item	4	8	12	16	CV (%)	Mean
Breathing rate (breaths/min)	82.68	80.47	85.64	80.03	9.74	82.21
Heart rate (beats/min)	103.83	105.08	101.07	104.50	6.78	103.62
Rectal temperature (°C)	39.62	39.45	39.49	39.51	0.32	39.52

[1] Means followed by different letters in a same row are statistically different (Tukey test, P<0.05). CV = coefficient of variation.

Table 6 - Average daily weight gain and feed efficiency of lambs in different levels of green propolis extract in experimental diets

Item	Green propolis extract level[1] (mL/day)				CV (%)	P
	4	8	12	16		
Average daily gain (g/day)[1]	139.81	147.95	131.41	111.04	26.87	0.00980
Feeding efficiency[2]	16.22	18.05	15.47	12.86	24.43	0.00020

EA = ADG/DMI*100; (P<0.05)
1 $Y = 123.219 + 6.21095*n - 0.439454n^2$ ($R^2 = 0.97$)
2 $Y = 13.50000 + 1.00938*n - 0.0664063n^2$ ($R^2 = 0.93$)

in energy efficiency caused by the propolis extract in the diet. It appears that from level 8 mL, there was reduction in GMD and deterioration of feed efficiency, which is possibly related to the action of flavonoids on inclusions of 4 and 8 mL, with ionophore activity in ruminal environment.

On the other hand, the levels of 12 and 16 mL possibly resulted in an excess of flavonoids in ruminal environment, what may have impaired bacteria and protozoa by toxicity, causing major losses of energy in the process of rumen degradation with negative impacts on ADG and feed efficiency. Van Soest (1994) affirmed that the animal response to a feed depends on complex interactions between diet composition, its processing, and consequently, the nutritional value, which is defined by three components: dry matter intake and digestibility and energy efficiency. This corroborates Leopoldino et al. (2007), who verified that antibiotics were effective in reducing the volume of gases, with the greater action of propolis, and reduction of methane, which resulted in lower power loss to the environment, in a ionophore evaluation by the techniques of cellular potassium loss and gas production *in vitro*.

Deriving the equations of regression (Table 6) for average weight gain and feed efficiency in function of level of the green propolis extract in the diet, there are points of maximum 7.07 mL and 7.60 mL per animal/day, respectively, for daily weight gain and feed efficiency.

Conclusions

Different levels of green propolis extract in the diet of lambs in confinement did not influence ingestive behavior, nutrient digestibility or physiological parameters. To maximize efficiency, the inclusion of 7.60 mL (2.1189 mg of dry matter and 0.1123 mg of flavonoids) of green propolis extract /day in the diet of confined lambs is recommended.

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