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Influence of solid residue from alcoholic extraction of brown propolis on intake, digestibility, performance, carcass and meat characteristics of lambs in feedlot

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ABSTRACT. Propolis can be an alternative to the use of ionophores in ruminants due to presence of compounds like flavonoids, fenolic acids, esters, fenolic aldehydes and ketones. In this study the impact of solid residue from alcoholic extraction of brown propolis (RBP) added to the diet for feedlot lambs on nutrients intake and digestibility, productive performance, body morphometric measures, carcass characteristics and meat fatty acid profile was determined. Twenty-four Texel × Suffolk crossbred castrated male lambs (19.83 ± 2.07 kg) were fed diet with 0 (negative control), 5, 10 g RBP per kg dry matter (DM) or monensin (positive control, 26 mg/kg DM), in total ration with roughage:concentrate ratio of 400:600 (w/w). RBP addition regardless used dose positively influenced ($P < 0.05$) productive performance parameters (final weight and daily weight gain). However, diet with 5 g RBP/kg DM lowered feed conversion ratio in comparison to negative control. Nutrient intake, digestibility of DM (684 g/kg), organic matter (701 g/kg) and fibre (695 g/kg) were similar among treatments. Live body length (78.89 cm), external carcass length (75.4 cm) and carcass compactness (0.253 kg/cm) also did not differ between groups. Moreover, diet supplementation with RBP did not affect hot and chilled carcass yields (451 and 447 g/kg, respectively). The addition of 5 g RBP/kg DM caused lower concentration of stearic acid (C18:0) and higher of oleic (C18:1n-9) and linoleic acids (C18:2n-6) in *longissimus* muscle of lambs in comparison to muscle from animals fed diet with monensin or control one. So, RBP can be used as nutritional additive to lamb feed to increase animal performance and modify meat fatty acid profile.

Introduction

Ionophores can inhibit Gram-positive bacteria and their inclusion into ruminant diet decreases acetate:propionate ratio by the decrease in hydrogen use for methane production, and ammonia-N levels and lactate production; moreover ionophores can increase protein availability, maintain rumen pH and decrease feed intake (Russell and Strobel, 1989). On the other hand, the use of ionophores such as sodium monensin has been associated with antimicrobial resistance in animals (Mathew et al., 2001). According to Ríspoli et al. (2009), the use of such substances is prohibited in some countries, therefore other compounds, as propolis or its residues of alcoholic extraction, are studied to replace them.

According to Mirzoeva et al. (1997) and Gomes et al. (2016), propolis exhibits bacteriostatic activity inhibiting Gram-positive and some Gram-negative bacteria by an apparent modification in the bioenergetic status of bacterial membrane and motility inhibition. This activity is primarily related to the flavonoid and phenolic acid content of propolis (Funari and Ferro, 2006). The content of these components, however may vary according to the ecology of the plants visited by bees (Ghisalberti, 1979). The composition of the ruminal microbiota is diet-dependent, and the fermentation of substrates and the efficiency of microbial synthesis may be strongly influenced by dietary changes (Lock et al., 2013). Propolis has been studied as an alternative to antibiotic additives to alter the ruminal microbiota, which could benefit fermentation processes and reduce biohydrogenation. Propolis extract caused a reduction in the *in vitro* growth of *Butyrivibrio fibrisolvens* (de Aguiar et al., 2013), main cellulolytic rumen bacteria. Yoshimura et al. (2018) examined the influence of diet supplementation with a propolis-based product on bacterial and protozoal populations in dairy cows and observed that the propolis-based product in diet tended to enhance *Butyrivibrio fibrisolvens* count but did not influence digestibility, short-chain fatty acid concentrations, pH and ammonia content in the rumen.

We have previously evaluated different forms of propolis (brown or green in crude, solid residue and alcoholic solutions) as an additive to ruminant feed. Effects have been found on the digestibility and conversion efficiency of the feed and the performance of ruminant animals. Ítavo et al. (2011a) concluded that propolis extract can be used as feed supplement instead of monensin sodium in the diets of feedlot lambs. Ítavo et al. (2011b) found that including either sodium monensin or brown propolis extract in the diet of feedlot lambs improves feed efficiency. Unfortunately, propolis extract used also for therapeutic purposes in humans is much more expensive than monensin. Although, the inclusion of residues produced during propolis alcoholic extraction into ruminant diets is feasible as a source of flavonoids and phenolic acids with antimicrobial activity (Heimbach et al., 2014; 2016; Gomes et al., 2016). Ítavo et al. (2009) found that dietary addition of brown propolis extract does not affect carcass characteristics of feedlot lambs. Likewise, da Silva et al. (2014) studied brown propolis in crude or extract forms as a feed supplement for feedlot lambs to identify the type mostly improving *in vivo* nutrient digestibility. They observed that the addition of brown propolis as crude (solid) or alcoholic extract forms exerts the same effect as monensin, when 500:500 (w/w) Tifton-85 hay:concentrate ratio in diet was used, but neither maximized nutrient availability in the diet of feedlot lambs at 7 months of age. In another work, da Silva et al. (2019) studied nutritional efficiency of the balanced supply of flavonoids from crude or ethanol extract of brown propolis on behaviour, productive performance and carcass traits of lambs in feedlot. The authors concluded that brown propolis supplementation can influence lamb carcass traits and meat quality; however, the form of propolis is an important factor.

Despite this, the effects of solid residue from alcoholic extraction of brown propolis (RBP) inclusion into lamb diets have not been studied yet. Therefore, it was hypothesized that RBP has the potential to replace as nutritional additive the sodium monensin, in association with a high quality feedlot lamb diet composed of 400:600 (w/w) roughage:concentrate ratio. The present study evaluated the effects of RBP inclusion into the diet on nutrients intake, productive performance, morphometric parameters of lambs and their carcasses, and meat fatty acid profile.

Material and methods

The experiment was carried out at the Federal University of Mato Grosso do Sul (UFMS) in Campo Grande (Brazil). The adopted protocols were approved by the UFMS Animal Research Ethics Committee (Protocol No. 218/2009).

Lambs, experimental design and diets

In total, 24 weaned, castrated Texel × Suffolk crossbred lambs of similar age and initial average weight of 19.83 ± 2.07 kg were used to conduct the study. Upon weaning, all lambs received a 2-ml intramuscular injection of antibiotic (Coccifin, Ouro Fino Saúde Animal, Ouro Fino, Cravinhos, SP, Brazil) to prevent coccidiosis. Lambs received an anthelmintic treatment (Cydectin, Fort Dodge Saúde Animal Ltda., São Paulo, SP, Brazil) upon weaning and 28 days later. Immediately after weaning, lambs were randomly allotted to individual pens (1.5 m × 2 m). Pens were assigned randomly to four experimental diets: (1) diet with no feed additive – negative control; (2) diet with 5 g/kg of RBP; (3) diet with 10 g/kg of RBP and (4) diet with 26 mg of sodium monensin in the total mixed diet – positive control. The additives were included in the concentrate just before feeding.

Lambs were housed in sheds made from clay tiles, with a ceiling height of 2.5 m and concrete paving. Individual pens were equipped with wood slat floor, waterer and feed trough. Water and mineral salt were provided *ad libitum*.

Diet was formulated to meet the NRC (2007) requirements for finishing lambs with an average body weight of 20 kg, a potential gain of 200 g/day and an estimated dry matter (DM) intake of 1 kg/day (Table 1). Diet was isonitrogenous and isocaloric. Tifton-85 hay (*Cynodon* spp.) ground to pass through a 5-mm mesh sieve was used as roughage at a forage:concentrate ratio of 400:600 on a DM basis. The same basal diet was offered to all animals but with different additives according to assigned group. Diets were fed *ad libitum* at 08:00 and 16:00. Amounts offered and refused were weighed daily and registered for each pen to determine feed intake. Feed consumption was adjusted for 100 g/kg orts as fed.

Table 1. Chemical composition of roughage and concentrate

Indices	Roughage Tifton-85 hay	Concentrate[1]
Dry matter (DM), g/kg	886	836
Organic matter, g/kg DM	932	932
Crude protein, g/kg DM	148	244
Neutral detergent fibre, g/kg DM	748	276
Acid detergent fibre, g/kg DM	401	110
Ether extract, g/kg DM	19.6	24.7
Total carbohydrates, g/kg DM	764	663
Non-fibrous carbohydrates, g/kg DM	16.7	387

[1] ingredients: g/kg DM: ground corn 517, soybean meal 482, mineral premix 1 (g: Ca 70, P 48, S 0.75, Na 1; mg: Co 0.30, Cu 3.75, I 0.42, Mn 9, Se 0.12, Zn 27)

Chemical composition of solid residue from extraction of brown propolis (RBP)

RBP was obtained from crude brown propolis alcoholic extraction. The brown propolis was obtained from 70 apiaries planted with alecrim-do-campo (*Baccharis dracunculifolia*) and assa-peixe (*Vernonia polyanthes*). The extract was obtained by infusing 30 g of ground crude brown propolis into 100 ml of grain alcohol solution (v/v) for 10 days (Stradiotti Junior et al., 2004). The solid residue was separated from the extract by filtration.

Nutrient intake and digestibility

Dry matter of feed ingredients, diets, orts and faeces was determined by drying samples in an oven at 105 °C overnight (AOAC International, 2000; method no. 930.15). All samples were dried in a forced-air oven at 55 °C for 72 h and ground through a 1-mm mesh before analyses of N, ether extract, acid detergent fibre (ADF) and neutral detergent fibre (aNDF). Total N was determined with a Tecnal TE-036/1 (Tecnal, Piracicaba, Brazil) according to method no. 976.05 of the AOAC International (2000). Ether extract was conducted with Tecnal TE-044/1 following method no. 920.39 of the AOAC International (2000). Ash content was determined by incineration at 600 °C for 2 h in a muffle furnace (AOAC International, 2000; method no. 942.05) and the organic matter content was calculated as the difference between 100 and the percentage of ash. Determination of aNDF was performed using a heat stable α-amylase (Termamyl 120 L®, Sigma-Aldrich, St. Louis, MO, USA) and without sodium sulphite, and expressed inclusive of residual ash. ADF inclusive of residual ash and lignin concentrations was determined by solubilisation of cellulose with sulphuric acid (H_2SO_4). Total carbohydrates were calculated by the equation: total carbohydrates = 1000 − (CP + ether extract + ash), while non-fibrous carbohydrates (NFC) were obtained using the equation: NFC = total carbohydrates − aNDF. The percentage of cellulose was obtained by the difference between ADF and lignin concentrations after sequential analysis.

Total content of polyphenols was measured colorimetrically in an aqueous extract using the Folin-Ciocalteu technique using polyvinylpolypyrrolidone for elimination of interfering substances. Polyphenols were extracted from RBP by mixing 1 g of sample (ground through a 1-mm screen) with methanol/water (90:10, v/v) and the volume was made up to 100 ml. The extracts were then filtered on a 0.22-μm PTFE membrane filter (Spritzen, Shanghai, China) in a tube protected from light. Concentration of polyphenols was expressed as gallic acid equivalents. Phytic acid concentration was determined by colorimetry. RBP was analysed to determine moisture level, mineral matter, ether extract, methanol-insoluble residue, flavonoids and total phenols (Table 2) as described by Funari and Ferro (2006).

Table 2. Chemical composition of solid residue from extraction of brown propolis

Indices	Content
Dry matter (DM), g/kg	888
Organic matter, g/kg DM	950
Ether extract (EE), g/kg DM	433
Wax, g/kg EE	172
Crude protein, g/kg DM	146
Total phenols, g/kg DM	24
Total flavonoids, g/kg DM	35

Samples of diets and orts were collected weekly, kept at −20 °C and pooled by pen and treatment for nutrient intake determinations. Composite samples of diets and orts were dried at 55 °C for 48 h and ground through a 1-mm screen in a Wiley mill (Marconi MA340, Piracicaba, SP, Brazil) for further chemical composition analyses.

Total collection of faeces was conducted from day 10 to day 13, day 20 to day 23, day 30 to day 33 and from day 40 to day 43 of the feeding experiment. Faeces were collected by fitting all lambs with a harness. Samples of feed and orts were taken daily during the faeces collection and pooled on a 8-day basis for further analyses, with one pooled sample of feed and orts analysed for each treatment and each pen, respectively. Daily output of faeces was determined, and all faeces were frozen at −20 °C and accumulated for the period. Thereafter, faecal samples were thawed and mixed thoroughly; a subsample was collected, freeze-dried and ground to pass a 1-mm screen using a Wiley mill for later chemical composition analysis.

Productive performance and *in vivo* body measures

Lambs were weighed at the beginning of the experiment and every 2 weeks during the 56-day experiment. Feed was withheld for 16 h before weighing the lambs.

In vivo measures were taken at the end of the experiment using a tape measure and measuring stick: *in vivo* body length (from the base of the tail to the base of the neck), height at withers and height at croup (based on the highest point above the ground), croup width and chest girth (around the back of the shoulders, next to the armpits) (Santana et al., 2001). The measures, in centimetres, were used to calculate body compactness index (WS per unit of body length).

Carcass measurements

After 56 days, lambs were fasted for 24 h and shipped to a commercial slaughterhouse. Lambs were knocked unconscious using electro narcosis of 220 V for 10 s. The carcass was split into two identical longitudinal halves and weighed to determine hot carcass weight. All carcasses were cooled at 4 °C for approximately 24 h and then take from the cooling chambers and weighed again in order to determine cold carcass weight. The difference between the chilled carcass weight and hot carcass weight was used to calculate carcass shrink loss. Internal and external carcass length, perimeter of the croup, and depth of the chest were determined on cold carcasses as described by Osório et al. (1996a, 1996b). In the left half of each carcass, commercial cut yield was measured by separating the carcass half into shoulder, fore shank loin, neck, rib, leg, and hind shank (Cartaxo et al., 2009) and the loin was separated into T-bone, tenderloin filet and rack. The palette was the sum of shoulder and fore shank, and the gammon was the sum of leg plus hind shank. Weight of each cut was divided by total weight of the cool carcass to obtain the retail yield. One sample also was collected from the cross section between the 9th and 13th rib on the left side of the carcass and dissected into lean, fat and bone tissues; each component was expressed as a percentage of the total weight of the rib sample to estimate physical composition. Subcutaneous fat was measured using a calliper rule on the left side of the carcass between the 12th and 13th ribs at the carcass midline. The *longissimus* muscle area between the 11th and 13th rib was drawn on plastic paper and measured using a planimeter (AUTOCAD® software, Autodesk, Inc, São Rafael, CA, USA). The *longissimus* muscle was completely removed from the left side of each carcass and frozen at −8 °C. Thereafter, three 25-mm thick steak samples were cut from each frozen sample of the *longissimus* muscle, weighed into aluminium trays and thawed for 24 h at 4 °C to obtain drip loss. Steaks then were used to determine cooking loss. Cooking was performed in an oven at 170 °C until a final cooking temperature of 71 °C was attained in core of the sample as determined with individual thermocouples metal drilling inserted in the geometric centre of each sample. Samples were then removed from the oven and the meat samples were blotted and weighed for the calculation of cooking losses (American Meat Science Association, 2015). Four 1-cm^2 cross sectional cores were prepared per sample with their lengths paralleled to the fibre axis. Shear force measurements were carried out with a Warner-Bratzler device using a Texture analyser, model CT3-25k (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) at 5 mm/s. Cores were sheared across the fibre axis by a V-shaped cutting blade with a triangular aperture of 60° and a shearing velocity of 200 mm/min (American Meat Science Association, 1995). Another set of three muscle samples was kept at −80 °C and freeze-dried. Dried meat samples were crushed in ball mill for proximate composition analysis. Methods of the AOAC International (2000) were used for determinations of moisture (no. 930.15), mineral matter (no. 942.05), protein (no. 976.05) and fat (no. 920.39)

contents. The organic matter portion was obtained by the difference between 1000 g/kg and mineral matter. The moisture was calculated by the difference between 1000 g/kg and the content of DM.

Fatty acid profile of *longissimus* muscle

Intramuscular fat from muscle samples was extracted. Freeze-dried samples of meat (4 g) were homogenized in 25 ml of methanol and 5 ml of chloroform using a tissue homogenizer set at 540 *g* (Model Q220 Quimis, Diadema São Paulo, SP, Brazil) for 30 min. The extracts were evaporated under 55 °C and lipids were stored at −80 °C until methylated. Sodium methoxide (10 ml), acetic acid (1 ml) and heptane (10 ml) were added to the mixture prior to a second homogenation carried out for 60 min. Samples were allowed to settle and 2 ml of lipids were collected from the upper heptane phase.

Fatty acids were methylated using sodium methoxide in methanol (1:25) as an agent of esterification and methyl acetate (1 ml) plus heptane (10 ml) to minimize saponification. Fatty acid methyl esters were quantified by gas chromatography (Agilent Technologies GC, model 6890N Network 237 GC System, Santa Clara, CA, USA) using a HP-88 capillary column (100 m × 0.25 mm i.d., 0.20 µm film thickness). The column parameters were as follows: initial temperature of 160 °C was maintained for 1 min; the temperature was then programmed at 6 °C/min to 230 °C and this temperature was maintained for 23 min. Injector and detector temperatures were 225 °C and 285 °C, respectively, and the volume of injection was 2 µl. The carrier gas was helium with flow 1.5 ml/min. Hydrogen flow to the detector was 35 ml/min, airflow was 450 ml/min, and the flow of N_2 make-up gas was 30 ml/min. Identification of fatty acids was done by comparison with the retention times of pure methyl ester standards (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

All data were submitted to analysis of variance (ANOVA) using the GLM procedure of SAS Software (2002; SAS Institute, Inc., Cary, NC, USA) according to a completely randomized design with four treatments. Data were analysed with the statistical model:

$$Y_{ij} = m + A_i + e_{ij},$$

where: Y_{ij} – j^{th} observation of the additive i; m – overall mean; A_i – effect of the additive associated to diet i; i – 1, 2, 3 and 4; and e_{ij} – random error associated to each Y_{ij} observation. Main source of variation was nutritional treatment (additive). Data on carcass measurements were analysed using slaughter weight as covariant. Feed intake and the feed:gain ratio were analysed using each lamb as the experimental unit and as well as lamb was the experimental unit for data on digestibility, performance and carcass. Comparisons of means were made using Tukey's adjustment for the probability. Significance was declared at $P \leq 0.05$ and a trend at $0.05 < P < 0.10$, unless otherwise stated.

Results

The addition of RBP into lamb feed influenced ($P < 0.05$) final body weight, total weight gain, daily weight gain (Table 3) and feed conversion ratio (Table 4). The final body weight and daily weight gain were higher in animals fed diet supplemented with RBP regardless used RBP dose in comparison to control animals. Feed conversion ratio was lower in animals fed diet with 5 g of RBP/kg DM in comparison to animals fed only basal diet. There were no significant effects of examined additives on *in vivo* measures: body length (78.9 cm), height at withers (56.87 cm), croup height (58.7 cm), croup width (22.8 cm), chest girth (77.7 cm) and body compactness index (0.4 kg/cm) of live lambs.

The RBP treatments did not influence ($P > 0.05$) DM (992.4 g/day), NDF (604.5 g/day) and metabolizable energy (2.4 Mcal/day) intakes. Similarly DM (684.1 g/kg), OM (701.1 g/kg) and NDF (695.0 g/kg)

Table 3. Productive performance and *in vivo* body measures of feedlot lambs fed diets with solid residue from extraction of brown propolis (RBP) addition

Indices	Additive				SEM	*P*-value
	0 (negative control)	5 g RBP/kg DM	10 g RBP/kg DM	monensin (25 mg/kg DM, positive control)		
Initial weight, kg	20.0	20.0	19.8	19.4	0.76	0.842
Final weight, kg	30.2^{b}	30.7^{a}	30.9^{a}	29.9^{b}	1.01	0.001
Total weight gain, kg	10.1^{b}	10.2^{b}	11.0^{a}	10.5^{ab}	0.24	0.001
Daily weight gain, g/day	188^{b}	203^{a}	204^{a}	200^{a}	1.45	0.001
Body length, cm	77.9	79.6	79.6	78.5	3.35	0.001
Withers height, cm	54.8	57.5	58.2	57.0	3.95	0.577
Croup height, cm	57.8	58.6	59.0	59.5	2.81	0.798
Croup width, cm	23.4	22.0	22.4	23.3	2.00	0.637
Chest girth, cm	76.6	76.8	79.9	77.5	4.63	0.659
Body compactness index, kg/cm	0.4	0.4	0.4	0.4	0.04	0.962

SEM – standard error of mean; ^{ab} – means with different superscripts within the same row are significantly different according to Tukey's post hoc test ($P < 0.05$)

Table 4. Nutrient intake and digestibility of feedlot lambs fed diets with solid residue from extraction of brown propolis (RBP) addition

Indices	Additive				SEM	*P*-value
	0 (negative control)	5 g RBP/kg DM	10 g RBP/kg DM	monensin (25 mg/kg DM, positive control)		
Intake						
DM, g/day	1003	974	1043	949	26.08	0.421
DM, g/kg BW	39.7	38.3	40.9	38.7	1.60	0.229
organic matter	951	924	989	899	23.10	0.258
crude protein	210	204	218	198	22.15	0.295
ether extract	23.1	22.5	24.1	21.9	2.05	0.278
neutral detergent fibre intake, g/day	611	594	635	577	1.17	0.394
neutral detergent fibre intake, g/kg BW	23.8	22.9	24.5	23.2	2.00	0.370
Digestibility						
DM, g/kg	691	662	700	683	8.42	0.306
organic matter, g/kg	706	682	716	700	8.30	0.398
crude protein, g/kg	755	716	769	752	8.17	0.086
neutral detergent fibre, g/kg	706	666	715	693	8.11	0.091
total digestible nutrient, g/kg	675	649	680	670	8.35	0.448
Metabolizable energy, Mcal/day	2.5	2.3	2.6	2.3	0.10	0.083
Feed conversion ratio, g/day of DM intake/g/day of weight gain	5.3^{b}	4.8^{a}	5.0^{ab}	4.7^{a}	0.57	0.003

DM – dry matter; BW – body weight; SEM – standard error of mean; ^{ab} – means with different superscripts within the same row are significantly different according to Tukey's post hoc test ($P < 0.05$)

digestibility and total digestible nutrient (668.6 g/kg) were not influenced by RBP addition (Table 4).

Carcass yield characteristics were not influenced by the RBP addition into diet: slaughter weight (30.3 kg), hot carcass weight (13.7 kg) and yield (451.2 g/kg), chilled carcass weight (13.6 kg) and yield (447.1 g/kg), subcutaneous fat thickness (2.3 mm), loin eye area (12.4 cm^2) (Table 5). Similarly, bone/carcass (0.2), muscle/carcass (0.5) and fat/carcass (0.3) proportions (Table 5), and also carcass measures and proportion of carcass cuts (Table 6) were not influenced by the examined additives.

There was significant effect (or tendency) of RBP addition into lamb diet on level of heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) in *longissimus* muscle (Table 7). Heptadecanoic acid level (C17:0) was lower in the meat of lambs fed diets containing 10 g RBP/kg DM than in those fed diets with monensin. The addition of 5 g RBP/kg DM caused the lower concentration of stearic acid (C18:0) and higher concentration of oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) in meat in comparison to meat from animals fed control diet or diet with monensin addition.

Table 5. Carcass characteristics of feedlot lambs fed diets with solid residue from extraction of brown propolis (RBP) addition

Indices	Additive				SEM	*P*-value
	0 (negative control)	5 g RBP/kg DM	10 g RBP/kg DM	monensin (25 mg/kg DM, positive control)		
Slaughter weight, kg	30.2	30.7	30.9	29.5	3.81	0.941
Hot carcass weight, kg	13.6	14.2	13.8	13.2	1.71	0.819
Hot carcass yield, g/kg	449.4	461.8	447.8	445.7	1.24	0.210
Cold carcass weight, kg	13.5	14.1	13.6	13.1	1.72	0.823
Cold carcass yield, g/kg	445.9	459.1	441.2	442.4	1.26	0.139
Losses from chilling, g/kg	7.6^{b}	5.8^{b}	14.9^{a}	7.5^{b}	0.58	0.011
Subcutaneous fat thickness, mm	2.6	2.3	2.3	2.2	0.64	0.873
Loin eye area, cm^2	12.1	12.0	13.1	12.6	0.53	0.628
Bone/Carcass proportion	0.2	0.2	0.2	0.2	0.06	0.773
Muscle/Carcass proportion	0.4	0.5	0.5	0.5	0.05	0.604
Fat/Carcass proportion	0.3	0.3	0.3	0.3	0.06	0.256

SEM – standard error of mean; ^{ab} – means with different superscripts within the same row are significantly different according to Tukey's post hoc test ($P < 0.05$)

Table 6. Carcass measures and proportion of carcass cuts of feedlot lambs fed diets with solid residue from extraction of brown propolis (RBP) addition

Indices	Additive				SEM	*P*-value
	0 (negative control)	5 g RBP/kg DM	10 g RBP/kg DM	monensin (25 mg/kg DM, positive control)		
Carcass measures						
external length, cm	73.4	77.3	73.9	77.0	2.96	0.110
internal length, cm	52.8	53.9	53.5	53.6	3.16	0.954
croup girth, cm	30.2	32.0	30.7	30.7	2.21	0.621
chest depth, cm	18.8	18.4	18.4	19.6	1.12	0.323
carcass compactness index, kg/cm	0.25	0.26	0.26	0.24	0.03	0.851
Proportion of carcass cuts						
gammon	0.33	0.32	0.32	0.34	0.01	0.151
palette	0.19	0.18	0.18	0.19	0.01	0.231
rack	0.16	0.17	0.16	0.16	0.01	0.643
rack cap off	0.16	0.15	0.15	0.14	0.01	0.384
loin	0.16	0.17	0.16	0.17	0.02	0.910
neck	0.06	0.06	0.06	0.06	0.01	0.345

SEM – standard error of mean

Table 7. Fatty acid (FA) profile (g/100 g of fatty acid methyl esters) of *longissimus* muscle of feedlot lambs fed diets with solid residue from extraction of brown propolis (RBP) addition

Fatty acids	Additive				SEM	*P*-value
	0 (negative control)	5 g RBP/kg DM	10 g RBP/kg DM	monensin (25 mg/kg DM, positive control)		
C10:0	0.15	0.13	0.13	0.16	0.023	0.131
C12:0	0.18	0.15	0.16	0.13	0.040	0.211
C14:0	3.25	3.02	2.40	2.99	0.383	0.443
C15:0	0.31	0.31	0.21	0.32	0.033	0.364
C16:0	28.13	28.48	26.37	27.89	1.344	0.610
C16:1	1.08	1.98	1.55	1.80	0.228	0.325
C17:0	1.00	1.11	0.93	1.17	0.067	0.095
C17:1	0.46	0.63	0.46	0.59	0.058	0.153
C18:0	17.51^{ab}	10.76^{b}	19.10^{a}	18.21^{a}	2.023	0.038
C18:1n-9	41.43^{b}	46.55^{a}	43.62^{ab}	40.41^{b}	1.540	0.046
C18:2n-6	2.40^{b}	2.75^{a}	1.98^{b}	2.69^{a}	0.192	0.048

SEM – standard error of mean; ^{ab} – means with different superscripts within the same row are significantly different according to Tukey's post hoc test ($P < 0.05$)

Discussion

The solid residue from alcoholic extraction of brown propolis (RBP) is hypothesised to possess the potential to replace the sodium monensin as beneficial nutritional additive in ruminant diet. As propolis extract is widely used by people because of its suggested therapeutic properties, the propolis extraction solid residue can be a cheaper beneficial by-product used in animal nutrition. So in the present study, RBP feed inclusion effects on nutrients intake, productive performance, assessment of morphometric parameters of lambs and their carcasses, and meat fatty acid profile were examined.

There was stated the RBP influence on lamb performance (final weight, total and daily weight gains and feed conversion ratio); however no effect of RBP on morphometric measurements of live lambs was observed. Likewise, Zawadzki et al. (2011), who evaluated the effects of three diets: control, containing sodium monensin and containing propolis extract on feedlot-finished bulls, stated that the average final weight and average daily gain were higher in bulls fed diet with propolis. They found also that carcass conformation, carcass length, leg length, cushion thickness, *longissimus* muscle area, *longissimus* muscle area/100 kg live weight, fat thickness, colour, texture, marbling,

pH and fragmentation index were not influenced by the examined treatments. The higher final weight observed in the present study and in the study of Zawadzki et al (2011) may occur due to several bioactive components in RBP like flavonoids (Olagaray and Bradford, 2019).

In the present study, there was also found no effect of RBP addition on nutrient intake and digestibility. In the previous study of our research group, da Silva et al. (2014) tested brown propolis in crude or extract form as a feed supplement using 500:500 (w/w) roughage (Tifton-85 hay):concentrate in diet and observed that lambs fed diets with crude propolis had higher feed intake than those fed diets containing monensin sodium. However, in the present study RBP addition into diet had similar effects to monensin on nutrients intake and digestibility. Ítavo et al. (2011a) assessed the productive performance of lambs finished in feedlot receiving diets supplemented with green propolis, brown propolis or monensin sodium and reported that lambs fed diets with either brown propolis or monensin sodium had lower nutrient intake in comparison to the control treatment.

According to Ítavo et al., (2011a) propolis is an alternative to the use of ionophores in ruminant nutrition due to presence of flavonoids, fenolic acids, esters, fenolic aldehydes and ketones (Funari and Ferro, 2006). The combination of the factors related to the floristic and ecological composition affects the pharmacological properties of propolis, which can be differently classified as brown, green and red propolis. According to Ítavo et al. (2011a) the green propolis is derived from 'alecrim-do-campo' (*Baccharis dracunculifolia*) with oxidation level lower than 10 g/kg, 279.9 g/kg of dry matter and flavonoid content (m/m) of 14.9 g/kg; and the brown propolis is derived from combination of 'alecrim-do-campo' (*B. dracunculifolia*) and 'assa-peixe' (*Vernonia polyanthes*) with oxidation level lower than 150 g/kg, 199 g/kg of dry matter and 4.5 g/kg of flavonoid content. The propolis exhibits bacteriostatic activity which is primarily related to the flavonoid and phenolic acid content of propolis (Mirzoeva et al. 1997; Funari and Ferro, 2006), and the content of these components may vary according to the ecology of the plants visited by bees (Ghisalberti, 1979). Gomes et al. (2017) evaluated *in vitro* fermentation characteristics of ruminant diets using ethanol extract of brown propolis as a nutritional additive and found that the degradation and fermentation of ruminant diet can be improved by using 13 ml/DM kg of ethanol extract of propolis containing 14 mg/ml of flavonoids. These divergences possibly occurred due to the content of flavonoid in the brown propolis extraction residue. According to de Aguiar et al. (2013) the growth of the strains of *Clostridium aminophilum* and *Fibrobacter succinogenes* was highly affected by propolis extracts. The propolis extracts inhibited the growth of *Fibrobacter succinogenes*, *Ruminococcus flavefaciens, Ruminococcus albus, Butyrivibrio fibrisolvens, Prevotella albensis, Peptostreptococcus sp., Clostridium aminophilum* and *Streptococcus bovis*. It was shown an increase in the lag phase and a decrease in the growth rate and yield with propolis, suggesting a bacteriostatic effect.

The digestibility of DM was not affected by treatments in the present study. Neither Ítavo et al. (2011a; 2011b) who examined influence of green or brown propolis alcoholic solutions nor da Silva et al. (2014) who examined crude propolis extract found differences in the nutrient digestibility, which was also confirmed in the present study. Likewise, Yoshimura et al. (2018) studying supplementation of diets with a propolis-based product observed that propolis-based product in diet tended to enhance *Butyrivibrio fibrisolvens* count but did not influence digestibility.

Da Silva et al. (2014) did not detect any effect of crude brown propolis or its extract on the biometrics or slaughter weight of feedlot lambs. Similarly, da Silva et al. (2019) did not observed effect of additives on the *in vivo* morphometric measurements and slaughter weight (35.3 kg). In addition, hot carcass weight (16.5 kg), cold carcass weight (15.0 kg), and cooling losses (88.6 g/kg) were not affected by the dietary addition of crude form or ethanol extract of brown propolis as nutritional additive for lambs in feedlot. The same in the present study – the addition of RBP did not affect hot carcass weight and chilled carcass weight. Hot carcass yield with average value of 451.2 g/kg was similar to one stated by Ítavo et al. (2009) in a study on lambs fed diets with added brown propolis (449 g/kg); however losses from cooling were lower in the present study, leading to higher chilled carcass yield (447.2 vs 418.5 g/kg in Ítavo et al. (2009)).

Subcutaneous fat thickness, on average 2.35 mm, did not differ among the treatments in the present study. Also, according to Ítavo et al. (2009), the dietary addition of propolis did not affect subcutaneous fat thickness. However, in some studies lower values of subcutaneous fat thickness were found. Ítavo et al. (2016) who studied the effect of different levels of crambe meal in the diet as a substitute for soybean meal on feed intake, growth performance, and carcass characteristics of lambs, found subcutaneous fat thickness at the level of 1.38 mm for male lambs and 2.09 mm for females. Similarly, Lima et al. (2018)

observed 1.30-mm subcutaneous fat thickness in the study conducted to determine the impact of sunflower cake inclusion in the diets of Santa Ines lambs on intake, performance and carcass characteristics.

The proportion of bone, muscle and fat in the carcass was not affected by the treatments. There were higher bone proportion and lower muscle and fat contents than that found by da Silva et al. (2014) (0.20, 0.49 and 0.31, respectively). Likewise, there was no effect of additive on external carcass length (75.4 cm) and it was slightly lower than the 78.75 cm reported by da Silva et al. (2014).

In the present study, the use of RBP, regardless used dose, did not affect the yield of commercial meat cuts. In the study on the addition of crude propolis or propolis extract to the diet of feedlot Texel crossbred lambs, da Silva et al. (2014) found yield values of 0.32 for leg, 0.18 for shoulder + shank and 0.15 for rack, which are similar to those obtained in the present study 0.31, 0.17 and 0.14, respectively.

The results obtained in the present study showed that RBP addition altered fatty acid profile of lamb meat. The RBP supplementation (at a dose of 5 g/kg DM) lowered the content of saturated fatty acids (SFA, such as stearic acid (C18:0)) increasing monounsaturated fatty acids content (oleic acid (C18:1n-9)) and polyunsaturated ones (linoleic acid (C18:2n-6)) in *longissimus* muscle in comparison to meat from animals fed control diet or diet with monensin addition. From all examined 11 fatty acids the highest contents were noted for: oleic acid, palmitic acid and stearic acid. Madruga et al. (2005) observed similar meat quality in Santa Inês lambs. This is likely related to the nature of this odd chain fatty acid, derived from microbial fats synthetized by bacteria as a function of propionate and valerianicum levels (Mansbridge and Blake, 1997). Flavonoids present in propolis as nutritional additive can increase the proportion of rumen propionate, thereby increasing C17:0 levels. However, in the present study, RBP at lower dose did not affect C17:0 level in relation to the control treatment and when higher dose was used the C17:0 content was even lower than in monensin or control groups.

Da Silva et al. (2019) examined the nutritional efficiency of the balanced supply of flavonoids from the crude or ethanol extract of brown propolis on carcass traits of lambs in feedlot and observed decrease effect of propolis on SFA content and an increase effect on unsaturated fatty acid (UFA) content in lamb meat in comparison to treatment without additive. Palmitic and stearic fatty acids appeared in the highest proportion among SFA.

Oleic acid (C18:1n-9), the most abundant unsaturated fatty acid, was higher in lambs receiving diets containing 5 g BPR/kg DM than in those receiving a control diet and a diet added with monensin. Similarly, da Silva et al. (2019) found that among the identified fatty acids oleic acid was the UFA that mostly contributed to the composition of the lipid profile. Given that serum cholesterol is affected by fatty acid composition and that oleic acid is said to decrease blood cholesterol (Madruga et al., 2005), it can be stated that the addition of RBP improved the fatty acid profile of lamb meat which is important for the consumer point of view.

Conclusions

The solid residue from alcoholic extraction of brown propolis (RBP) can be used as nutritional additive in feedlot lamb diet. The addition of RBP, especially at a dose of 5 g/kg DM, not only improved the growth performance of animals, but also positively influenced fatty acid profile of lamb meat by decreasing the content of saturated fatty acid and increasing the content of unsaturated ones.

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