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Effect of dietary addition of phenolic compounds from propolis on growth performance, carcass traits, and meat fatty acid profile of feedlot beef cattle

Efeito da adição de compostos fenólicos da própolis na ração sobre o desempenho produtivo, características de carcaça e perfil de ácidos graxos da carne de bovinos confinados

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Highlights

Propolis-based products (PBP) have been studied as additives for ruminant nutrition.
Two PBP dosages containing 225 and 300 mg g^{-1} phenolic compounds were tested.
Feed conversion ratio was better with PBP containing 225 mg g^{-1} of phenolic compounds.
Both dosages maintained meat redness better than the control after 9 days at 4 oC.
No major changes were observed in the fatty acid profile of the meat with the PBP.

Abstract

Twenty-four male Nellore steers (445 ± 31 kg initial body weight) were used to evaluate the effects of different doses of phenolic compounds from a propolis-based product (PBP) on growth performance, carcass traits, and meat fatty acid (FA) profile. The total mixed ration consisted of 470 g kg^{-1} corn silage and 530 g kg^{-1} concentrate (dry matter [DM] basis), which were randomly assigned to one of three treatments: control (CON, without phenolic compounds from the propolis extract), PBP1 (225 mg of phenolic compounds g^{-1} of propolis dry extract), and PBP2 (300 mg of phenolic compounds g^{-1} of propolis dry extract). The animals were fed in a feedlot for 84 days and presented an average final body weight (FBW) of 542 kg. Dietary addition of phenolic compounds had no overall effect on growth performance. Mean values for dry matter intake, average daily gain and feed conversion ratio were 9.99 kg d^{-1}, 1.14 kg d^{-1} and 8.82 kg

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DM kg gain^{-1}, respectively. Hot carcass weight and hot carcass yield had average values of 308 kg and 56.8%, respectively. In addition, carcass traits did not change after PBPs were added to the diet, except for the ribeye area, which was higher for PBP2 (21.5 cm^2 100 kg^{-1}) than for PBP1 (18.6 cm2 100 kg^{-1}). Dietary addition of PBPs maintained redness better than the controls after 9 days in the refrigerator; no major changes were observed in the meat FA profile after the addition of PBPs to the diet. These results suggest that phenolic compounds present in propolis (300 mg g^{-1}) have positive effects on meat color and improve the sensory quality of meat.
Key words: Artepillin C. Phenolic. Meat color. Meat quality. Plant extract. Propolis extract.

Resumo

Foram utilizados vinte e quatro novilhos da raça Nelore (445 ± 31 kg de peso corporal inicial) para avaliar o efeito de diferentes doses de compostos fenólicos obtidos a partir de produto a base de própolis (PBP) sobre o desempenho produtivo, características de carcaça e perfil de ácidos graxos (AG). A ração total foi constituída em 470 g kg^{-1} de silagem de milho e 530 g kg^{-1} de concentrado (com base na matéria seca) e os animais foram distribuídos nos seguintes tratamentos: controle (CON, sem compostos fenólicos do extrato seco de própolis), PBP1 (225 de compostos fenólicos g^{-1} de extrato seco de própolis) e PBP2 (300 mg de compostos fenólicos g^{-1} de extrato seco de própolis). Os animais foram alimentados em confinamento por 84 dias e apresentaram peso corporal final médio de 542 kg. A adição dos compostos fenólicos da própolis na ração não influenciou o desempenho produtivo. Os valores médios para consumo de matéria seca, ganho médio diário e conversão alimentar foram 9,99 kg d^{-1}, 1,14 kg d^{-1} e 8,82 kg MS kg ganho^{-1}, respectivamente. O peso de carcaça quente e o rendimento de carcaça quente apresentaram valores médios de 308 kg e 56,8%; respectivamente. Além disso, as características da carcaça não foram alteradas após a adição dos PBPs à dieta, exceto para a área de olho de lombo, que foi maior para o tratamento PBP2 (21,5 cm^2 100 kg^{-1}) quando comparada ao PBP1 (18,6 cm^2 100 kg^{-1}). A adição dos PBPs manteve a carne mais vermelha do que o CON após 9 dias na geladeira; não foram observadas grandes alterações no perfil de AG da carne após a adição dos PBPs à dieta. Os resultados sugerem que os compostos fenólicos presentes na própolis (300 mg g^{-1}) têm efeitos positivos na coloração da carne, melhorando sua qualidade sensorial.
Palavras-chave: Artepillin C. Coloração da carne. Extrato de planta. Extrato de própolis. Qualidade da carne.

Introduction

In recent years, consumer concerns about the quality of the animal products they consume have increased. They want healthy products from production systems that care about the environment, welfare, and animal health (Popa et al., 2018). In ruminants, it is possible to meet consumer demands by manipulating the rumen and its microbes, and the use of antibiotic ionophores has been highly successful in improving feed efficiency, animal productivity, and quality of animal products. However, the emergence and spread of antimicrobial resistance have become a major public health problem in the European Union and worldwide. Therefore, instead of synthetic feed additives and

antibiotics, research should focus on the use of natural products, medicinal and aromatic plants, or similar products in animal nutrition (Manav et al., 2020).

Propolis, or bee glue, is a natural wax-like resinous substance found in bee hives and is used by honeybees as a cement to seal cracks or open spaces (Kuropatnicki et al., 2013). It contains phenolic compounds that are responsible for the antimicrobial (Aguiar et al., 2013) and antioxidant effects (Silva Frozza et al., 2013) of propolis. Some studies have shown that propolis is effective in increasing the intestinal digestibility of crude protein, reducing ruminal ammonia nitrogen (NH_3-N) production (Aguiar et al., 2014a), and improving the productive performance of lambs (Ítavo et al., 2019). Furthermore, propolis inhibits in vitro total gas production (Araújo et al., 2018), reduces ruminal ciliate protozoa in buffaloes (Paula et al., 2016), and inhibits the growth of certain rumen bacterial strains in vitro (Aguiar et al., 2013, 2014b). However, despite these findings, little is known about the effects of propolis on food products (meat and milk) obtained from these animals.

Meat consumers demand products of higher and consistent quality, with a distinctive flavor and aroma, and those that can provide a particular sensory experience when consuming beef (Álvarez et al., 2021). Substances derived from plants have been shown to reduce lipid oxidation in meat products, with antioxidant properties related to their phenolic content. Although synthetic antioxidants are widely used in the meat industry to reduce losses due to oxidation, consumer concerns over their toxicity have prompted the search for natural sources of antioxidants (Castillo et al., 2013). Therefore, the objective of this study was to investigate the effects of different doses of phenolic compounds from a propolis-based product (PBP) on the growth performance, carcass traits, and meat fatty acid (FA) profile of feedlot beef steers.

Material and Methods

The research was carried out in Maringá City (23 ° 25' 38" S, 51 ° 56' 15" W, 555 m altitude), Paraná, Brazil. Propolis samples were obtained from the apiary at the Experimental Farm of Iguatemi, Universidade Estadual de Maringá, Paraná State, Brazil. The apiary is located within a reserve of eucalyptus (*Eucalyptus* sp.) surrounded by a native forest containing alecrim-do-campo (*Baccharis dracunculifolia*). The PBP is protected in Brazil by an intellectual property application, registered with number 0605768-3.

Twenty-four male Nellore steers (initial body weight [BW]: 445 ± 31 kg) with an average age of 30 months were housed in individual pens (10 m^2) with free access to water. The animals were randomly assigned to the following treatments: Control (CON; without phenolic compounds from the propolis extract), PBP1 (224.73 mg of phenolic compounds g^{-1} of propolis dry extract), and PBP2 (299.64 mg of phenolic compounds g^{-1} of propolis dry extract).

The dry propolis extracts were obtained from concentrations of propolis that ranged from 5.0 to 30.0 g in 100 mL of a water-alcohol solution (60.0-93.8 mL of alcohol) by turbo extraction for 15 minutes. The extract was filtered under vacuum, the alcohol was removed (<15%) using a rotary evaporator, and the extract was dried in a lyophilizer (Liobras®, model K105, São Carlos, SP, Brazil)

at - 40 °C for 12 h. The PBP powder fed to the animals contained dry propolis extract and an excipient (ground corn). The excipient was used to add volume to the propolis dry extract and facilitate feeding. The composition of phenolic compounds found in the dry extract of propolis is presented in Table 1. The quantification of these compounds was performed using high-performance liquid chromatography with a photodiode array detector (Alliance HPLC-PDA, Waters Co., Milford, MA, USA). The total mixed ration (TMR, Table 2) consisted of 470 g kg^{-1} corn silage and 530 g kg^{-1} concentrate (dry matter [DM] basis), and was formulated to meet the requirement of 1.4 kg of average daily gain (ADG) (National Research Council [NRC], 2000). All animals were fed the same TMR, which differed only with the addition of propolis (PBP). PBP was added to the feed at the time of feeding, and the doses were adjusted with 10 g of excipient (5 g of PBP at 08:00 h and 5 g at 16:00 h). PBP was provided at the time of feeding and before mixing the concentrate with the silage to ensure the intake of the amounts of propolis stipulated in each treatment.

Table 1
Composition of flavonoids and phenolic acids identified in the propolis dry extracts

	Propolis dry extract[I]		
	Initial dose	PBP1	PBP2
Phenolic compounds (mg g^{-1} of propolis dry extract)			
Chlorogenic acid	0.72	2.16	2.88
Caffeic acid	8.52	25.56	34.08
ρ-coumaric acid	18.21	54.63	72.84
CAPE[II]	2.96	8.88	11.84
Artepillin C	11.38	34.14	45.52
Apigenin	5.59	16.77	22.36
Pinocembrin	7.49	22.47	29.96
Galangin	2.69	8.07	10.76
Chrysin	6.63	19.89	26.52
Acacetin	10.72	32.16	42.88
Total phenolic compounds	74.91	224.73	299.64

[I]PBP1: 225 mg of phenolic compounds g^{-1} of propolis dry extract, equivalent to three times the initial dose; PBP2: 300 mg of phenolic compounds g^{-1} of propolis dry extract, equivalent to four times the initial dose.
[II]Caffeic acid phenethyl ester.

Table 2
Ingredients and chemical composition of the total mixed ration

	Total mixed ration (TMR)
Ingredients (g kg DM^{-1})	
Corn silage	470.0
Ground corn	434.0
Soybean meal	72.9
Mineral premix[a]	17.5
Limestone	3.8
Ammonium sulfate	1.8
Chemical composition (g kg DM^{-1})	
Dry matter (g kg^{-1} as fed)	635.7
Crude protein	131.0
Ether extract	28.3
Neutral detergent fiber	317.0
Acid detergent fiber	164.0
Total carbohydrates	815.0
Non-fiber carbohydrates	497.0
Organic matter	961.8
Total digestible nutrients	729.0
TMR energy partition (Mcal kg DM^{-1})	
Digestible energy	3.20
Metabolizable energy	2.60

[a] Composition of the mineral premix (per kg of product): 90 g of Ca, 65 g of P, 145 g of Na, 4.69 g of S, 60 mg of I, 1050 mg of Mg, 10 mg of Se, 2880 mg of Zn, 80 mg of Co, 1200 mg of Cu, 1500 mg of Fe.

During the experiment, TMR samples were collected every 28 days and analyzed to determine their chemical composition. The analyses to determine DM (method 934.01), organic matter determined by ash (OM, method 924.05), crude protein (CP, method 920.87), and ether extract (EE, method 920.85) in samples milled to 1 mm were conducted in accordance with Association of Official Analytical Chemists [AOAC] (1990). The contents of neutral detergent fiber (aNDF) and acid detergent fiber (ADF), both inclusive of residual ash, were analyzed following the procedures of Van Soest et al. (1991), with amylase but without sodium sulfite in the neutral detergent solution. Total carbohydrates (TC) were calculated using the following equation: TC = 100 − (%CP + %EE + %ash) (Sniffen et al., 1992). Non-fiber carbohydrates (NFC) were calculated as the difference between TC and NDF. The total digestible nutrient (TDN) content of the

experimental diet was estimated using the following equation (NRC, 2000): %TDN = %DCP + %DNDF + %DNFC + %(DEE × 2.25), where DCP is the digestible crude protein, DNDF is the digestible neutral detergent fiber, DNFC is the digestible non-fibre carbohydrate, and DEE is the digestible ether extract. The TDN values were converted to digestible energy (DE) and metabolizable energy (ME) using the following equations (NRC, 2000): DE (Mcal kg^{-1}) = 0.04409 × TDN (%), and ME (Mcal kg^{-1}) = 1.01 × DE (Mcal kg^{-1})-0.45.

The following variables were recorded to evaluate growth performance: dry matter intake (DMI), initial BW (IBW), final BW (FBW), average daily gain (ADG), feed conversion ratio (FCR), and metabolizable energy conversion ratio (MECR). Feed intake was recorded daily, and the amount offered was adjusted to allow 100 g kg^{-1} of refusals to be fed. Daily feed intake was defined as the difference between the supplied feed and refusals in the trough. Samples of the diet (corn silage and concentrate) and the refused portion were taken during the experiment to determine the DM content, according to the AOAC (1990) method 934.01. IBW and FBW were determined by weighing the animals before and after the experimental period (84 days), respectively. All animals were fasted for solids for 14 h before weighing. The following variables were calculated: ADG = gain (FBW - IBW) feedlot $period^{-1}$ (28, 56, and 84 days); FCR = DMI (kg d^{-1})/ADG (kg); and MECR = MEI (Mcal kg^{-1})/ADG (kg), where MEI is the metabolizable energy intake.

At the end of the experimental period (84 days), the animals were slaughtered at a commercial slaughterhouse, 20 km away from the experimental farm. The hot carcass weight was obtained, and the carcasses were stored in a cold chamber (approximately 2 °C) for 24 h. After cooling, a fraction of the *Longissimus thoracis et lumborum* (LTL) muscle, located between the 10^{th} and 12^{th} ribs, was removed from the right-half of each carcass to be used in physical and chemical tests. Hot carcass yield was calculated as follows: hot carcass yield (%) = (hot carcass weight × 100) FBW^{-1}. Carcass pH was measured in LTL on the left side of the carcass using a Sentron portable pH meter (Gig Harbor, WA, USA). The ribeye area between the 12^{th} and 13^{th} thoracic vertebrae was measured using a vegetal paper to trace the perimeter of the LTL. Fat thickness was measured using a caliper between the 12^{th} and 13^{th} ribs over the LTL and averaged over three points. Marbling, texture, and color were evaluated using the Brazilian scoring system (Müller, 1987). Two individuals independently assessed the amount of marbling fat on the cut surface of the eye muscle on both sides of the carcasses. Texture was determined by fascicle size and was subjectively evaluated. Muscle color was evaluated on 24-h chilled carcasses, 30 min after a cross-sectional cut was made on the LTL, between the 12^{th} and 13^{th} ribs.

Samples of the LTL were cut in transverse slices (steaks) 2.5-cm thick, wrapped in aluminum foil, and stored in polyethylene bags at -20 °C. After freezing, one meat sample was taken from the freezer, unwrapped, weighed, placed in a refrigerator at 4 °C for 24 h for thawing, and weighed again to determine thawing losses. The meat samples were then cooked on an electric grill until their internal temperature reached 40 °C, obtained using a contact thermometer (IKA®, model ETS-D5, Campinas, SP, Brazil); then they

were turned over and cooked until the internal temperature was 71 °C, and then cooled at room temperature and weighed again to determine cooking losses. Warner-Bratzler shear force measurements were performed according to Wheeler et al. (2007) using a texturometer (model TA-XT2i, Stable Micro System Ltd., Godalming, Surrey, UK) coupled to a Warner-Bratzler Shear force probe. Briefly, each cooked steak (sub-sample) was cut into four parallelepiped-shaped pieces, so that the blade (probe) of the texturometer transversely cut the muscle fibers in a 3-cm^2 face, and the average of the four replicates was calculated as the SI Newton units (1 g = 0.009806 N) required to break the fibers cm^{-3}.

To determine meat color, a CR-400 Minolta Chroma Meter (Minolta Corporation/ ISD, Ramsey, NJ, USA) with a 0.8-cm aperture, a D65 light source, and a 2° observer was used to measure lightness (L*), redness (a*), and yellowness (b*) according to the CIELAB system (Commission Internationale de l'Éclairage [CIE], 1986). For this purpose, another meat sample of *M. LTL* was thawed in a refrigerator at 4 °C for 24 h and then split into four equal sub-samples with a diameter of 8 cm. One subsample was analyzed for color, and the other three subsamples, properly identified, were placed in trays lined with absorbent paper, covered with aluminum foil, and maintained at 4 °C in a refrigerator. After 3, 6, and 9 days, the sub-samples were evaluated for color, as previously described.

The LTL samples taken at the 10^{th} and 11^{th} ribs were ground, homogenized, and analyzed in triplicate. Total lipids were extracted using the method described by Bligh and Dyer (1959) using a chloroform/methanol mixture. Fatty acid methyl esters (FAMEs) were prepared by triacyl glycerin methylation according to the ISO method (International Organization for Standardization [ISO], 1978). The FAMEs were separated and quantified using a Thermo Scientific gas chromatograph (model Trace GC Ultra; Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an autosampler TriPlus flame ionization detector and fused silica capillary column CP Sil-88 (100 m, 0.25 mm i.d., and 0.25 µm cyanopropyl polysiloxane). The gas flows (White Martins, Praxair Technology Inc., Danbury, CT, USA) were 1.2, 30, 35, and 350 mL $minute^{-1}$ for the carrier gas (H_2), auxiliary gas (N_2), H_2, and synthetic air flame, respectively. The injector and detector temperatures were 230 and 250 °C, respectively. The column temperature used to separate the FAMEs was programmed at 165 °C for 18 min, followed by a first ramp of 4 °C $minute^{-1}$ up to a maximum of 170 °C, at which the temperature was maintained for 9.5 minutes. Injections of 2 µL were performed for all samples with a 1:80 split ratio. Peak areas were determined using ChromQuest software (version 5.0), and FAs were identified by comparing the relative retention times of the FAME peaks of the samples with a FAME standard 189-19 (Sigma Company, St Louis, MO, USA) by spiking samples with the standard. The results are expressed as g 100 g^{-1} of total fatty acids.

The experiment was conducted using a completely randomized design. The individual steer was the experimental unit (three treatments with eight animals each [n=8]) for each animal (DMI, IBW, FBW, ADG, FCR, MECR carcass traits, instrumental meat quality, and meat FA profile). For intake and BW, statistical analyses were based on the data averaged over the entire experimental period

of 84 days. The statistical model contained the effect of the treatment (fixed term) in the following manner:

$$Y_{ij} = \mu + T_i + e_{ij},$$

where Y_{ij} is response j in treatment i, μ is the overall mean, Ti is the fixed effect of treatment i, e_{ij} is the random error. Data were analyzed using the general linear model procedure in SAS (Statistical Analysis System, version 9.0). Differences between the treatment means were determined using Tukey's test. Tests that returned a value of $P < 0.05$ were considered statistically significant.

Results and Discussion

The addition of phenolic compounds from PBP to the diet did not affect growth performance compared to CON (Table 3); however, there were significant differences between the dosages of PBP for FBW, ADG, FCR, and MECR. Animals in the treatment containing the highest dose of phenolic compounds (PBP2) had a significantly lower FBW (P = 0.030) and ADG (P = 0.008) than those in the PBP1 treatment (the lowest dose of phenolic compounds), as well as a significantly higher FCR at the beginning (28 days; P = 0.004) and end (84 days; P = 0.009) of the feedlot period. The PBP1 treatment group exhibited the greatest weight gain during the 84-day feedlot period (P = 0.030) and significantly improved FCR (P = 0.009) and MECR (P = 0.010) compared to the PBP2 treatment. Although PBPs did not affect growth performance compared to CON, a higher dose of phenolic compounds (PBP2) impaired growth performance compared to the lower dose (PBP1). The ADG of steers in the PBP2 treatment group was 27% lower than that in the PBP1 treatment group. In addition, the FCR in the PBP2 treatment increased by 116% and 48% at the beginning and end of the feedlot period, respectively. Weight gain was 26% higher in the PBP1 group than in the PBP2 group after the 84-day feedlot period. There were no significant differences in DMI among treatments, indicating that the poor growth performance exhibited by the PBP2 treatment was not due to the amount of feed and nutrients ingested, but to the dosage of phenolic compounds added to the diet.

The use of different doses of PBPs in the diet of beef cattle has been investigated previously. Zawadzki et al. (2011a) reported higher FBW, ADG, and FCR for feedlot-finished bulls fed PBP1 (the same propolis extract used in this experiment, but with three times lower dosage) than those fed the control and monensin. Likewise, Valero et al. (2014a) evaluated the effects of propolis and essential oils on the growth performance of feedlot-finished bulls and found higher FBW, ADG, and gain:feed ratio for propolis and essential oils than for controls. Propolis exhibits antimicrobial activity against rumen bacteria in vitro (Aguiar et al., 2013), but its mechanism of action has not been elucidated. However, studies have shown that propolis can reduce ruminal N-NH3 production and improve nitrogen (N) utilization by ruminants (Oeztuerk et al., 2010; Ozturk et al., 2010). In addition, propolis seems to enhance individual, total short-chain fatty acids and acetate:propionate ratio, as well as reduce methane emission (Morsy et al., 2021). These results do not corroborate with those obtained in the present study, since, in general, propolis did not differ from controls in terms of growth performance. However, because propolis is not a "standardized" component and its

composition depends on the type, amount of propolis, and solvents used for its extraction, there are divergent results regarding its use as an additive in animal nutrition. This hinders the interpretation of the results because the biological properties of propolis are directly related to the amount and composition of phenolic compounds present in the extracts (Toreti et al., 2013).

Table 3
Performance of feedlot Nellore finishing cattle fed diets with or without phenolic compounds of propolis

Items[I]	Treatments[II]			SEM	p-value
	CON	PBP1	PBP2		
DMI (kg d^{-1})	9.99	9.90	10.10	0.58	0.960
DMIBW (g kg BW^{-1})	21.10	20.4	21.4	1.1	0.790
IBW (kg)	443.13	447.62	443.89	11.66	0.960
FBW (kg)	541.10^{ab}	558.1^{a}	527.34^{b}	6.92	0.030
ADG (kg)					
0 - 28 d	1.25	1.51	0.88	0.17	0.060
0 - 56 d	1.25	1.40	1.05	0.10	0.090
0 - 84 d	1.16^{ab}	1.31^{a}	0.96^{b}	0.07	0.008
FCR (kg DM kg $gain^{-1}$)					
0 - 28 d	8.22^{a}	6.49^{a}	14.01^{b}	1.45	0.004
0 - 56 d	8.02	6.88	10.21	0.93	0.058
0 - 84 d	8.36^{ab}	7.31^{a}	10.81^{b}	0.74	0.009
Total gain (kg)	98.00^{ab}	110.50^{a}	81.25^{b}	6.81	0.030
MECR (Mcal kg $gain^{-1}$)	21.06^{ab}	18.28^{a}	26.16^{b}	1.81	0.010

[I] DMI: dry matter intake; DMI_{BW}: DMI relative to BW; IBW: initial body weight; FBW: final body weight; ADG: average daily gain; FCR: feed conversion ratio. MECR: metabolizable energy conversion ratio.
[II] CON: without propolis dry extract; PBP1: 225 mg of phenolic compounds g^{-1} of propolis dry extract; PBP2: 300 mg of phenolic compounds g^{-1} of propolis dry extract.
Means followed by different lowercase letters in the same row differ by Tukey test ($P < 0.05$).

Carcass traits (Table 4) did not change after PBPs were added to the diet (P > 0.05), but different doses of phenolic compounds from the PBPs affected the ribeye area, which was significantly greater in the PBP2 treatment than in the PBP1 treatment (P = 0.001). The increase in the ribeye area in the PBP2 group was 15.6% greater than that in the PBP1 group, but did not differ from that in the CON group. However, few studies have investigated the effects of propolis and its phenolic compounds on carcass traits.

Zawadzki et al. (2011a) reported that feedlot Nellore bulls fed a PBP1 diet had significantly higher carcass weights than the controls and those fed monensin. Ítavo et al. (2019), also observed better FCR and weight gain in lambs fed with propolis extract. Silva et al. (2019) observed that carcass yield was lower in lambs fed diets with sodic monensin than in lambs fed diets with crude propolis. In addition, the authors verified that the addition of propolis extract to the diet decreased carcass yield compared to the control group, suggesting that the form of propolis used is an important factor. However, Valero et al. (2014a) found no effect of natural additives (propolis and essential oils) on carcass traits of feedlot crossbred bulls.

Table 4
Carcass traits of feedlot Nellore finishing cattle fed diets with or without phenolic compounds of propolis

Trait	Treatments[I]			SEM	*p*-value
	CON	PBP1	PBP2		
Hot carcass weight (kg)	307.00	319.00	298.00	6.21	0.070
Hot carcass yield (%)	56.7	57.2	56.5	0.5	0.660
Ribeye area (cm^2)	61.6	59.4	63.9	1.4	0.140
Ribeye area (cm^2 100 kg^{-1})	20.1[ab]	18.6[b]	21.5[a]	0.4	0.001
Fat thickness (mm)	6.64	8.21	6.71	0.92	0.410
Colour score[II]	4.07	3.71	3.86	0.23	0.540
Texture score[III]	4.00	4.00	4.29	0.20	0.540
Marbling score[IV]	6.29	6.86	8.57	1.76	0.640
pH	5.90	5.88	5.91	0.05	0.920

[I] CON: without propolis dry extract; PBP1: 225 mg of phenolic compounds g^{-1} of propolis dry extract; PBP2: 300 mg of phenolic compounds g^{-1} of propolis dry extract.
[II] 5 point scale, 1 - dark, 2 - dark red, 3 - slightly red, 4 - red, 5 - cherry red.
[III] 5 point scale, 1 - very coarse, 2 - coarse, 3 - slightly, 4 - fine, 5 - very fine.
[IV] 18 point scale, 1 to 3 - traces, 4 to 6 - light, 7 to 9 - small, 10 to 12 - mean, 13 to 15 - moderate, 16 to 18 - abundant.
Means followed by different lowercase letters in the same row differ by Tukey test ($P < 0.05$).

Regarding instrumental meat quality (Table 5), no differences were found in shear force and cooking losses among treatments ($P > 0.05$), but the PBP2 group had significantly lower thawing losses than the CON and PBP1 groups ($P = 0.030$). In addition, the PBP diet maintained redness better than the CON diet after 9 days in the refrigerator ($P = 0.005$, Table 5). The fat thickness (7.18 mm) complied with the Brazilian market guidelines. According to Müller (1987), fat must be at least 3-5 mm thick for good conservation and to prevent damage to the carcass during cooling; the values obtained in the present study were above the minimum required. Our results for color, texture, marbling, and pH were within the requirements of the Brazilian market, indicating good quality meat, regardless of treatment.

Table 5
Instrumental meat quality of feedlot Nellore finishing cattle fed diets with or without phenolic compounds of propolis

	Treatments[I]			SEM	*p*-value
	CON	PBP1	PBP2		
Shear force (kg cm^{-3})	3.27	3.13	3.27	0.20	0.850
Thawing loss (%)	10.70[b]	10.75[b]	8.24[a]	0.70	0.030
Cooking loss (%)	18.8	21.8	20.7	1.4	0.350
CIELAB color parameters	6.64	8.21	6.71	0.92	0.410
*L** values					
Thawed	34.82	34.36	34.23	0.71	0.825
3 days	37.04	37.14	36.49	0.85	0.845
6 days	35.65	35.32	35.92	1.12	0.932
9 days	36.68	36.82	35.84	0.92	0.720
*a** values					
Thawed	17.77	18.40	18.33	0.43	0.544
3 days	17.78	17.59	17.91	0.58	0.929
6 days	14.27	14.33	15.02	0.38	0.330
9 days	13.17[b]	15.01[a]	14.70[a]	0.36	0.005
*b** values					
Thawed	8.43	7.90	8.09	0.36	0.598
3 days	9.68	9.53	9.29	0.29	0.629
6 days	9.64	9.24	8.91	0.24	0.133
9 days	9.86	9.65	9.10	0.58	0.641

[I] CON: without propolis dry extract; PBP1: 225 mg of phenolic compounds g^{-1} of propolis dry extract; PBP2: 300 mg of phenolic compounds g^{-1} of propolis dry extract.
Means followed by different lowercase letters in the same row differ by Tukey test ($P < 0.05$).

The shear force, which measures meat tenderness, was not modified by adding phenolic compounds from PBP to the diet (Table 5); and however, there are no data regarding the effect of propolis and its phenolic compounds on instrumental meat quality, warranting further investigation. The average value obtained was 3.22 kg cm^{-3}, indicating soft meat (Wheeler et al., 2007).

Meat color has been reported as the most important factor for consumers when assessing meat quality because it relates color to freshness (Velasco & Williams, 2011). The two doses of PBP used in this study were able to maintain redness of the meat after nine days at 4 °C (Table 5). Polyphenols found in propolis are plant secondary metabolites that have been associated with health benefits such as antioxidant activity (Olagaray & Bradford, 2019). It is important to note that the main botanical source used to produce propolis in this study was *B. dracunculifolia*, and it has been demonstrated that many chemical substances present in this plant are also present in green propolis, such as flavonoids and coumaric acid derivatives (Aguiar et al., 2014c). Caffeic acid, p-coumaric acid, and artepillin C were found in large amounts in *B. dracunculifolia* (Guimarães et al., 2012), as was found in the propolis extracts used in this study (Table 1). Guimarães et al. (2012) demonstrated that the high concentration of phenolic compounds present in extracts of *B. dracunculifolia* confers a potent antioxidant effect through both free radical scavenging and iron chelating activities. It is probable that the phenolic compounds used in this study, such as caffeic acid, *p*-coumaric acid, and artepillin C, exerted antioxidant effects in the *LTL*, thus preventing myoglobin oxidation.

No major changes were observed in the meat FA profile with the addition of PBPs to the diet (Table 6); however, the amount of *cis*-5-14:1 (myristoleic acid) was higher in the PBP2 treatment group than in the CON and PBP1 groups ($P = 0.037$). Among the PBP treatments, the amounts of *cis*5-16:1 (11-hexadecanoic acid) and *cis*9-16:1 (7-hexadecanoic acid) increased ($P = 0.048$ and $P = 0.045$, respectively), with the highest increase observed in the PBP2 treatment. No differences were found between the treatment groups for MUFA, PUFA, and SFA ($P > 0.05$). The total CLA, n-3, and n-6 contents were not affected by the addition of PBPs to the diet ($P > 0.05$), as was the case for the n6:n3 ratio. Previous studies investigating the addition of PBP to the diet of feedlot beef cattle have also reported the effects of propolis on the FA profile of meat (Zawadzki et al., 2011b; Valero et al., 2014b); however, the results are divergent, which makes it difficult to understand the mechanism of action of propolis on rumen FA metabolism. Aguiar et al. (2013) reported that the same PBP used in this study exhibited antimicrobial activity *in vitro* against *Butyrivibrio fibrisolvens* (which dominates the microbial community in the biohydrogenation of FA), and there are still few studies on the influence of propolis on ruminant-derived products (meat and milk). Aguiar et al. (2014c) evaluated the effect of PBPs on the milk FA profile of middle-lactation dairy cows and observed that the milk FA profile was changed by the addition of PBP, which shows that propolis plays an important role in rumen microorganisms involved in the biohydrogenation process. The paucity of studies on phenolic compounds from propolis and ruminal metabolism, and their effects on meat quality and FA profile, hinder the interpretation of our results; therefore, further studies are necessary.

Table 6
Fatty acid profile (g 100 g^{-1} of total fatty acids) on the *Longissimus thoracis et lumborum* muscle of feedlot Nellore finishing cattle fed diets with or without phenolic compounds of propolis

	Treatments[I]			SEM	*p*-value
	CON	PBP1	PBP2		
14:0	3.15	2.56	3.17	0.25	0.167
*cis*5-14:1	0.12^{a}	0.12^{a}	0.15^{b}	0.00	0.037
*cis*7-14:1	0.87	0.68	0.81	0.06	0.118
15:0	0.29	0.30	0.34	0.01	0.072
16:0	27.06	26.54	27.75	0.55	0.321
*cis*5-16:1	0.45^{ab}	0.42^{b}	0.47^{a}	0.01	0.048
cis7-16:1	3.48	2.92	3.26	0.23	0.258
cis9-16:1	0.17^{ab}	0.16^{b}	0.18^{a}	0.00	0.045
17:0	0.85	0.95	0.93	0.03	0.204
*cis*9-17:1	0.63	0.62	0.60	0.02	0.824
18:0	14.30	14.89	14.78	0.60	0.757
*cis*9-18:1	41.70	43.17	41.14	1.03	0.383
*trans*9-18:1	1.34	1.31	1.27	0.08	0.860
*trans*9,*trans*12-18:2	0.46	0.42	0.40	0.03	0.459
*cis*6-18:2	2.78	2.74	2.57	0.27	0.853
*cis*3-18:3	0.35	0.35	0.35	0.02	0.969
*cis*6-18:3	0.09	0.09	0.09	0.00	0.777
*cis*9,*trans*11-18:2	0.34	0.34	0.41	0.03	0.177
*trans*10,*cis*12-18:2	0.07	0.08	0.08	0.00	0.069
*cis*6-20:3	0.26	0.23	0.22	0.02	0.655
*cis*6-20:4	0.83	0.72	0.67	0.09	0.516
*cis*3-20:5	0.24	0.22	0.20	0.02	0.586
*cis*3-20:6	0.09	0.07	0.06	0.01	0.205
MUFA[II]	48.50	49.20	47.70	1.17	0.670
PUFA[II]	5.51	5.29	5.09	0.43	0.780
SFA[II]	45.4	45.00	46.70	1.10	0.540
PUFA:SFA	0.12	0.12	0.10	0.01	0.646
Total CLA[III]	0.41	0.42	0.50	0.03	0.138
n-3	0.68	0.66	0.62	0.06	0.740
n-6	4.49	4.29	4.05	0.38	0.720
n6:n3	6.66	6.52	6.58	0.19	0.890

[I] CON: without propolis dry extract; PBP1: 225 mg of phenolic compounds g^{-1} of propolis dry extract; PBP2: 300 mg of phenolic compounds g^{-1} of propolis dry extract.
[II] MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.
[III] CLA: conjugated linoleic acid.
Means followed by different lowercase letters in the same row differ by Tukey test ($P < 0.05$).

Conclusion

Compared with a finishing feedlot diet without phenolic compounds of propolis, diets containing 225 and 300 mg of phenolic compounds per gram of propolis dry extract did not affect the growth performance and carcass traits of Nellore finishing cattle. The highest phenolic compound dosage used in this study reduced their growth performance in relation to the lowest level of this additive. Regarding instrumental meat quality, the phenolic compounds of propolis maintained redness after nine days, and no major changes were observed in the meat fatty acid profile.

Statement of animal rights

The experimental protocols that were developed in this study fully complied with the ethical principles of animal experimentation prepared by the Brazilian College of Animal Experimentation (COBEA) and were referred to the Ethics Committee on Animal Use in Experimentation, State University of Maringá, for consideration under approval number 019/2008.

Conflict of interest

The authors declare that they have no conflicts of interest, and this document is their original research work.

References

Aguiar, S. C., Cottica, S. M., Boeing, J. S., Samensari, R. B., Santos, G. T., & Visentainer, J. V. (2014c). Effect of feeding phenolic compounds from propolis extracts to dairy cows on milk production, milk fatty acid composition, and the antioxidant capacity of milk. *Animal Feed Science and Technology, 193*, 148-154. doi: 10.1016/j.anifeedsci.2014.04.006

Aguiar, S. C., Paula, E. M., Yoshimura, E. H., Santos, W. B. R., Machado, E., Valero, M. V., Santos, G. T., & Zeoula, L. M. (2014a). Effects of phenolic compounds in propolis on digestive and ruminal parameters in dairy cows. *Revista Brasileira de Zootecnia, 43*(4), 197-206. doi: 10.1590/S1516-35982014000400006

Aguiar, S. C., Zeoula, L. M., Franco, S. L., Peres, L. P., Arcuri, P. B., & Forano, E. (2013). Antimicrobial activity of Brazilian propolis extracts against rumen bacteria in vitro. *World Journal Microbiology & Biotechnology, 29*, 1951-1959. doi: 10.1007/s11274-013-1361-x

Aguiar, S. C., Zeoula, L. M., Prado, O. P. P., Arcuri, P. B., & Forano, E. (2014b). Characterization of rumen bacterial strains isolated from enrichments of rumen content in the presence of própolis. *World Journal of Microbiology & Biotechnology, 30*, 2017-2926. doi: 10.1007/s11274-014-1719-8

Álvarez, S., Mullen, A. M., Hamill, R., O'Neill, E., & Álvarez, C. (2021). Chapter three - dry-aging of beef as a tool to improve meat quality. Impact of processing conditions on the technical and organoleptic meat properties. *Advances in Food and Nutrition Research, 95*, 97-130. doi: 10.1016/bs.afnr.2020.10.001

Araújo, C. M., Silva, F. G. B., Castro, D. P. V., Menezes, D. R., Queiroz, M. A. A., & Yamamoto, S. M. (2018). Gas production and in vitro degradability of sheep diets containing própolis ethanolic extract.

Revista Brasileira de Saúde e Produção Animal, 19(3), 277-286. doi: 10.1590/S1519-99402018000300005

Association of Official Analytical Chemists (1990). *Official methods of analysis* (15nd ed.). AOAC.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extration and purification. Canadian *Journal of Biochemistry and Physiology, 37*(8), 911-917. doi: 10.1139/o59-099

Castillo, C., Pereira, V., Abuelo, A., & Hernández, J. (2013). Effect of supplementation with antioxidants on the quality of bovine milk and meat production. *The Scientific World Journal, 2013*, 1-8. doi: 10.1155/2013/616098

Commission Internationale de l'Éclairage (1986). *Colorimetry* (nº. 15.2, 2nd ed.). CIE Publications.

Guimarães, N. S. S., Mello, J. C., Paiva, J. S., Bueno, P. C. P., Berretta, A. A., Torquato, R. J., Nantes, I. L., & Rodrigues, T. (2012). Baccharis dracunculifolia, the main source of green propolis, exhibits potent antioxidant activity and prevents oxidative mitochondrial damage. *Food and Chemical Toxicology, 50*(3-4), 1091-1097. doi: 10.1016/j.fct.2011.11.014

International Organization for Standardization (1978). *Animal and vegetable fats and oils - preparation of methyl esters of fatty acids. Method ISO 5509.* ISO.

Ítavo, C. C. B. F., Ítavo, L. C. V., Esteves, C. A. T., Sapaterro, G. A., Silva, J. A., Ferelli, K. L. S. M., & Souza Arco, T. F. F. (2019). Influence of solid residue from alcoholic extraction of brown propolis on intake, digestibility, performance, carcass and meat characteristics of lambs in feedlot. *Journal of Animal and Feed Sciences, 28*(2), 149-158. doi: 10.22358/jafs/109284/2019

Kuropatnicki, A. K., Szliszka, E., & Krol, W. (2013). Historical aspects of propolis research in modern times. *Evidence-based Complementary and Alternative Medicine, 2013*, 1-11. doi: 10.1155/2013/964149.

Manav, S., Yilmaz, M., Baytekin, H., Çelik, K., & Çağli, A. (2020). The use of propolis as an antimicrobial in livestock - an overview. *Agricultural Science and Technology, 12*(3), 205-209. doi: 10.15547/ast.2020.03.032

Morsy, A. S., Soltan, Y. A., El-Zaiat, H. M., Alencar, S. M., & Abdalla, A. L. (2021). Bee propolis extract as a phytogenic feed additive to enhance diet digestibility, rumen microbial biosynthesis, mitigating methane formation and health status of late pregnant ewes. *Animal Feed Science and Technology, 273*, 1-11. doi: 10.1016/j.anifeedsci.2021.114834

Müller, L. (1987). *Normas para avaliação de carcaças e concurso de carcaça de novilhos.* UFSM.

National Research Council (2000). *Nutrients requirements of beef cattle* (7nd rev. ed.). National Academy of Science.

Oeztuerk, H., Emre, B., Sagmanligil, V., Piskin, I., Fidanci, U. R., & Pekcan, M. (2010). Effects of nisina and propolis on ruminal fermentation in vitro. *Journal of Animal and Veterinary Advances, 9*(21), 2752-2758. doi: 10.3923/javaa.2010.2752.2758

Olagaray, K. E., & Bradford, B. J. (2019). Plant flavonoids to improve productivity of ruminants - a review, *Animal Feed Science and Technology, 251*, 21-36. doi: 10.1016/j.anifeedsci.2019.02.004

Ozturk, H., Pekcan, M., Sireli, M., & Fidanci, U. R. (2010). Effects of propolis on in vitro rumen microbial fermentation. *Ankara Üniversitesi Veteriner Fakültesi Dergisi, 57*, 217-221. doi: 10.1501/Vetfak_000000 2428

Paula, E. M., Samensari, R. B., Machado, E., Pereira, L. M., Maia, F. J., Yoshimura, E. H., Franzolin, R., Faciola, A. P., & Zeoula, L. M. (2016). Effects of phenolic compounds on ruminal protozoa population, ruminal fermentation, and digestion in water buffaloes. *Livestock Science, 185*, 136-141. doi: 10.1016/j.l ivsci.2016.01.021

Popa, M. E., Mitelut, A., Popa, E. E., & Stan, A. (2018). Organic foods contribution to nutritional quality and value. *Trends in Food Science & Technology, 84*, 15-18. doi: 10.1016/j.tifs.2018.01.003

Silva Frozza, C. O., Celi Garcia, C. S., Gambato, G., Oliveira de Souza, M. D., Salvador, M., Moura, S., Padilha, F. F., Seixas, F. K., Collares, T., Borsuk, S., Dellagostin, O. A., Pegas Henriques, J. A., & Roesch-Ely, M. (2013). Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. *Food and Chemical Toxicology, 52*, 137-142. doi: 10.1016/j.fct.2012.11.013

Silva, J. A., Ítavo, C. C. B. F., Ítavo, L. C. V., Graça Morais, M. da, Silva, P. C. G. da, Ferelli, K. L. S. M., & Souza Arco, T. F. F. de. (2019). Dietary addition of crude form or ethanol extract of brown própolis as nutritional additive on behaviour, productive performance and carcass traits of lambs in feedlot. *Journal of Animal and Feed Sciences, 28*(1), 31-40. doi: 10.22358/jafs/105442/2019

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science, 70*(11), 3562-3577. doi: 10.2527/1992.70113562x

Toreti, V. C., Sato, H. H., Pastore, G. M., & Park, Y. K. (2013). Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evidence-based Complementary and Alternative Medicine, 2013*, 1-13. doi: 10.1155/2013/697390

Valero, M. V., Prado, R. M., Zawadzki, F., Eiras, C. E., Madrona, G. S., & Prado, I. N. (2014a). Propolis and essential oils additives in the diets improved animal performance and feed efficiency of bulls finished in feedlot. *Acta Scientiarum. Animal Sciences, 36*(4), 419-426. doi: 10.4025/actascianimsci.v36i4.23856

Valero, M. V., Torrecilhas, J. A., Zawadzki, F., Bonafé, E. G., Madrona, G. S., Prado, R. M., Passeti, R. A. C., Rivaroli, D. C., Visentainer, J. V., & Prado, I. N. (2014b). Propolis or cashew and castor oils effects on composition of Longissimus muscle of crossbred bulls finished in feedlot. *Chilean Journal of Agricultural Resaearch, 74*(4), 445-451. doi: 10.4067/S0718-58392014000400011

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Symposim: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. *Journal of Dairy Science, 74*(10), 3583-3597. doi: 10.3168/jds. S0022-0302(91)78551-2

Velasco, V., & Williams, P. (2011). Improving meat quality through natural antioxidants. *Chilean Journal of Agricultural Research, 71*(2), 313-322. doi: 10.4067/S0718-58 392011000200017

Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2007). Beef longissimus slice shear force measurement among steak locations and institutions. *Journal of Animal Science, 85*(9), 2283-2289. doi: 10.2527/jas.2006-736

Zawadzki, F., Prado, I. N., Marques, J. A., Zeoula, L. M., Prado, R. M. do, Fugita, C. A., Valero, M. V., & Maggioni, D. (2011b). Sodium monensin or propolis extract in the diet of Nellore bulls finished in feedlot: chemical composition and fatty acid profile of Longissimus muscle. *Semina: Ciências Agrárias, 32*(4), 1627-1636. doi: 10.5433/1679-0359.2011v32n4p1627

Zawadzki, F., Prado, I. N., Marques, J. A., Zeoula, L. M., Rotta, P. P., Sestari, B. B., Valero, M. V., & Rivaroli, D. C. (2011a). Sodium monensin or propolis extract in the diets of feedlot-finished bulls: effects on animal performance and carcass characteristics. *Journal of Animal and Feed Sciences, 20*(1), 16-25. doi: 10.223 58/jafs/66153/2011