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Feeding propolis or essential oils (cashew and castor) to bulls: performance, digestibility, and blood cell counts¤

Alimentación de toros con propóleos o aceites esenciales (anacardo y ricino): desempeño, digestibilidad y conteo de células sanguíneas

Alimentação de touros com própolis ou óleos essenciais (caju e mamona): desempenho, digestibilidade e contagem de células sanguíneas

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Summary

Background: in cattle production systems, antibiotics are commonly fed to cattle to prevent diseases, metabolic disorders, and to improve feed efficiency. Considerable effort has been devoted towards developing alternatives to the use of antibiotics. **Objective:** to evaluate the effect of propolis or cashew and castor oils on animal performance, feed intake, digestibility, and blood cell counts of young bulls. **Methods:** bulls were kept in a feedlot for 49 days. Starting on the 40th day, feces were sampled for five days to estimate digestibility using indigestible dry matter (DM) as a marker. Bulls were fed a control diet (CON) with sorghum silage (41% DM) and cracked corn, soybean meal, glycerine, limestone, and mineral salt. The propolis-supplemented group (PRO) received 3 g/animal/d in the concentrate. The essential oils-supplemented group (OIL) received 3 g/animal/d (1.5 g cashew oil + 1.5 g castor oil) added to the concentrate. **Results:** final body weight, average daily gain and feed efficiency were better for bulls fed the OIL diet. Propolis or essential oils had no effect on feed intake and digestibility. There was no effect of propolis or essential oils on blood cell counts. Red blood cell concentration was greater in the last day of the experiment, while the number of white blood cells was lower. **Conclusions:** dietary addition of propolis did not affect bull performance or feed efficiency. The

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addition of essential oils improved performance. Feed intake, digestibility, and blood cell counts were similar in all treatments.

Keywords: *antioxidants, biodiesel, cattle, co-products, feedlot, plant oils.*

Resumen

Antecedentes: en los sistemas de producción ganaderos, los antibióticos son añadidos a los bovinos para prevenir enfermedades y perturbaciones metabólicas y mejorar la eficacia alimenticia. Se han realizado considerables esfuerzos para desarrollar aditivos alternativos a los antibióticos. **Objetivo:** evaluar el efecto de los propóleos o aceites de anacardo y ricino sobre los índices productivos, ingestión de alimentos, digestibilidad y células sanguíneas de toros jóvenes. **Métodos:** los toros fueron mantenidos en un sistema de cebo intensivo durante 49 días. A partir del día 40, se tomaron muestras de heces durante cinco días para estimar la digestibilidad (utilizando como marcador la materia seca indigerible). Los toros fueron alimentados con una dieta de control (CON) con ensilaje de sorgo (41% de DM) y maíz triturado, harina de soja, glicerina, piedra caliza y sal mineral. El grupo suplementado con propóleos (PRO) recibió 3 g/animal/d en el concentrado. El grupo suplementado con los aceites esenciales (OIL) recibió 3 g/animal/d (1,5 g de anacardo + 1,5 g de aceite de ricino) añadido al concentrado. **Resultados:** el peso corporal final, promedio de ganancia diaria y eficacia alimenticia fueron mejores para los toros jóvenes alimentados con la dieta OIL. Los propóleos o aceites esenciales no tuvieron efecto sobre el consumo de alimento y digestibilidad. No hubo efecto de propóleos o la adición de aceites esenciales en las dietas con respecto a los valores medios de las células sanguíneas. El número de células rojas sanguíneas fue mayor en el último día de experimento, mientras que el número de células blancas sanguíneas fue menor. **Conclusiones:** la adición de propóleos en la dieta de los toros jóvenes no tuvo efecto ni en el desempeño ni la eficiencia alimenticia. La adición de aceites esenciales mejoró el desempeño animal. La ingestión de alimentos, digestibilidad y las células sanguíneas fueron similares en todos los tratamientos.

Palabras clave: *aceites de plantas,* antioxidante*, biodiesel, bovino, cebadero, co-productos*.

Resumo

Antecedentes: no sistema de produção de bovinos, os antibióticos são fornecidos aos bovinos para prevenir doenças e perturbações metabólicas e melhorar a eficiência alimentar. Consideráveis esforços têm sido realizados para desenvolver produtos alternativos aos antibióticos. **Objetivo:** avaliar o efeito de produtos alternativos: própolis, óleos de caju e mamona no desempenho animal, ingestão de alimentos, digestibilidade e células sanguíneas de toros jovens. **Métodos:** os animais foram confinados durante 49 dias. No quadragésimo dia de confinamento, as fezes foram amostradas por cinco dias para determinar a digestibilidade (matéria seca indigestível foi usada como indicador). Os animais foram alimentados com uma dieta controle (CON) com silagem de sorgo (41% da matéria seca) e milho moído, farelo de soja, glicerina, calcário e sal mineral. O grupo própolis (PRO) recebeu 3 g/animal/d dieta. O grupo com óleos essenciais (OIL) receberam 3 g/animal/d (1,5 g de óleo de mamona + 1,5 g de óleo de caju) na dieta. **Resultados:** o peso final, o ganho médio diário e a eficiência alimentar foram melhores para os animais alimentados com a dieta OIL. Própolis ou óleos essenciais não tiveram efeito na ingestão de alimentos e digestibilidade aparente. Não houve efeito de própolis ou dos óleos essenciais na dieta nos valores de células sanguíneas. A concentração de células vermelhas foi maior no último dia do experimento, enquanto que a concentração de células brancas foi menor. **Conclusões:** a adição de própolis na dieta dos animais em sistema intensivo de produção não teve efeito sobre o desempenho animal e eficiência alimentar. A adição de óleos essenciais melhorou o desempenho animal. A ingestão de alimentos, digestibilidade e células sanguíneas foram similares em todos os tratamentos.

Palavras chave: *antioxidante, biodiesel, bovino, confinamento, coprodutos, óleos de plantas*.

Introduction

In Brazil, grazing is the system used for raising beef cattle (Ferraz and Felício, 2010). It, therefore, becomes crucial to evaluate technological alternatives capable of increasing efficiency of the industry and, consequently, restructuring the beef production chain. Thus, due to increases in the consumption of beef and the demand for quality products by consumers in Brazil, finishing cattle in feedlots may be a tool to maximize production and improve meat quality (Prado *et al.*, 2008; 2012; Rotta *et al.*, 2009). In Brazil, bulls are finished in feedlots when they reach 380 Kg body weight (BW) at about 24 months of age (Maggioni *et al.*, 2009; Rotta *et al.*, 2009). There has been an increase in using feedlot systems for finishing young bulls at 18 months (Dian *et al.*, 2010; Ito *et al.*, 2010) and bulls after weaning at 10 and 12 months of age (Ito *et al.*, 2012). However, to maximize production efficiency of bulls finished in feedlots it is necessary to use diets with high energy density (NRC, 2000). To increase the energy density of the diet, it is necessary to use carbohydrate-rich cereals and co-products from the agri-food system (Marques *et al.*, 2000). Carbohydrates degrade rapidly, which can disturb ruminal fermentation (Giger-Reverdin *et al.*, 2002; Martins *et al.*, 1999). Therefore, some substances have been used to control ruminal fermentation, including antibiotics and other compounds (Zawadzki *et al.*, 2011). However, in recent years, public concern over routinary use of antibiotics in livestock nutrition has increased due to the emergence of antibiotic-resistant bacteria that may represent a risk to human health (Russell and Houlihan, 2003). Consequently, considerable effort has been devoted towards developing alternatives to antibiotics (Benchaar *et al.*, 2008; Valero *et al.*, 2011). Propolis and plant extracts offer an interesting opportunity in this regard (Zhang *et al.*, 2010; Zawadzki *et al.*, 2011).

Natural plant extracts contain a wide variety of compounds with different functions and mechanisms of action (Benchaar *et al.*, 2008; Zhang *et al.*, 2010). Phenolic compounds, terpenoids, essential oils, and polyacetylenes are among the plant compounds that have antimicrobial characteristics, all of which have particular mechanisms of action (Zhang *et al.*, 2010). Many plants produce secondary metabolites, which have properties to modulate ruminal fermentation (Benchaar *et al.*, 2008). Natural plant extracts and propolis have compounds –e.g. phenol- with bioactive properties such as anti-inflammatory, antioxidant, antiviral, and antiparasitic (Fischer *et al.*, 2008). Data on the effects of essential oils and their compounds on beef cattle performance are almost non-existent.

The aim of this study was to evaluate the effect of adding propolis or plant oils to the diets of growing bulls reared in an intensive system with corn and glycerine as energy sources on their performance, apparent digestibility and blood cell counts.

Materials and methods

Ethical considerations

This experiment was approved by the Department of Animal Production of Universidade Estadual de Maringá, Brazil (CIOMS/OMS, 1985) and conducted at the Rosa & Pedro Sector of Iguatemi Experimental Station Farm of the same university.

Thirty crossbred bulls (½ Aberdeen Angus, ½ Nellore) were used in a completely randomized design. Bulls were weighed and distributed into three diet groups with ten replications per group. Initial BW was 321 ± 27 Kg, with initial age of 18 ± 2 months old. Bulls were housed in individual pens on concrete floors with 10 m²/bull, equipped with feeders (60 cm deep and 2 m in length) and drinkers with a capacity of 250 L of water. The chemical composition of the feed is presented in Table 1.

The composition of diets is shown in Table 2. The intake of concentrate and sorghum silage was recorded daily until day 49 of the experimental period when bulls reached 387 ± 10.7 Kg BW.

Glycerine was produced in a soy-diesel facility (BIOPAR, Rolândia, Paraná, South of Brazil). The propolis product contains 0.054 mg/g total flavonoids in chrysin. The essential oils contain ricinoleic acid, anacardic acid, cardanol, and cardol. Ricinoleic acid was obtained from castor oil (extracted from castor seeds). Anacardic acid, cardanol, and cardol were obtained from cashew nut shell liquid (from the processing of cashew nuts).

Bulls were randomly assigned to one of three diets: CON – Control, PRO – propolis addition, and OIL – essential oils addition. Bulls were fed twice a day at 08:00 and 16:00 h. The diet formulation was designed to provide 1.4 Kg/d body weight gain, according to the NRC (2000) recommendations.

Daily feed intake was estimated as the difference between supplied feed and orts. Feed and orts samples were collected during the collection period and a representative composite sample was drafted per animal in each treatment. Bulls were weighed at the beginning of the experiment and every 14 days (after

Table 1. Chemical composition of ingredients and diets (g/Kg of dry matter).

Ingredients	g/Kg									
	DM[1]	OM[2]	Ash	CP[3]	EE[4]	NDF[5]	ADF[6]	TC[7]	NFC[8]	TDN[9]
Sorghum silage	260	937	62.5	54.6	26.7	665	426	856	190	540
Corn	875	988	11.7	93.4	33.5	154	49.3	861	707	900
Soybean meal	909	938	61.3	496	22.5	106	103	419	313	820
Glycerin	942	10.0	47.6	1.00	60.0					807
Urea	990			2620						
Mineral salt[10]	990		990							
Limestone	990		950							
Propolis	146									
Essential oils	976	559	440							

[1]Dry matter. [2]Organic matter. [3]Crude protein. [4]Ether extract. [5]Neutral detergent fiber. [6]Acid detergent fiber. [7]Total carbohydrates. [8]Non fibrous carbohydrates. [9]Total digestible nutrients. [10]Guarantee levels (per Kg) of calcium: 175 g; phosphorus: 100 g; sodium: 114 g; selenium: 15 g; magnesium: 15 g; zinc: 6,004 mg; manganese: 1,250 mg; copper: 1,875; iodine: 180 mg; cobalt: 125 mg; selenium: 30 mg; fluorine (maximum): 1,000 mg.

Table 2. Diets composition (g/Kg DM).

Ingredients	Diets		
	CON[1]	PRO[2]	OIL[3]
Sorghum silage	415	415	415
Corn	333	333	333
Soybean meal	80.7	80.7	80.7
Glycerin	153	153	153
Urea	8.20	8.20	8.20
Mineral salt	5.00	5.00	5.00
Limestone	5.00	5.00	5.00
Propolis		0.55	
Essential oils			0.55

[1]Control diet. [2]Diet with propolis inclusion. [3]Diet with essential oils inclusion.

fasting from solid feed for 16 hours) for the duration of the experiment to determine performance (49 days).

Fecal collections were performed over the course of five days, starting on the 40th day of the feedlot period, to estimate apparent total digestibility of DM and other nutrients. Fecal samples (approximately 200 g wet weight) were collected from each bull from the pen floor (minimum 3 h intervals between samples) over five consecutive days and were pooled per bull for each 5-day sampling period (Zeoula *et al.,* 2002). Samples were ground in a feed mill and passed through a 1-mm sieve after drying at 55 °C for 24 h (Nocek, 1985).

Indigestible dry matter (iDM) was used as an internal marker to estimate fecal DM (Zeoula *et al.*, 2002). Samples were milled through a 2 mm sieve, packed (5 mg DM/cm^2) in a 4 x 5 cm Ankom (filter bags F57 - ANKOM Technology, Macedon, NY, USA) that had been previously weighed, and then incubated for 240 h in the rumen of a Holstein bull (Casali *et al.*, 2008) that was fed a mixed diet of equal parts forage (sorghum silage) and concentrate (the same concentrate that was used in the treatments). After incubation, bags were removed, washed with water until clean, and dried in a forced-air oven at 55 °C for 72 h, after which they were removed and oven-dried again at 105 ºC. The iDM was estimated using the difference in sample weight before and after ruminal incubation. Fecal excretion was calculated with the following equations:

$$FE = iDMI / iDMCF$$

Where:

FE = fecal excretion (Kg/d).

iDMI = iDM intake (Kg/d).

iDMCF = iDM concentration in feces (Kg/d).

Apparent digestibility coefficients (ADC) for DM and nutrients were estimated according to the following formula:

$$DC = [(\text{Intake} - \text{Excreted}) / \text{Intake}] \times 100$$

The DM content of the ingredients (silage and concentrate), orts, and feces was determined after drying the mat at 105 °C for 16 h according to AOAC (1998; method 930.15). Organic matter (OM) content was calculated as the difference between DM and ash, with the ash content determined by combustion at 550 °C for 5 h according to AOAC (1998; method 930.15). Nitrogen (N) content was determined by the Kjeldahl method (AOAC, 1998; method 976.05). Neutral detergent fiber (NDF) content was determined using the methods described by Van Soest *et al.* (1991) and acid detergent fiber (ADF; AOAC, 1998; method 973.18). Total carbohydrates (TC) were estimated using the following equation (Sniffen *et al.*, 1992): TC = 100 – (% CP + % EE + % Ash). Non-fibrous carbohydrates (NFC) were determined as the difference between TC and NDF. Total digestible nutrient (TDN) content of diets was obtained with the methodology described by Kearl (1982).

Blood was collected twice: at the beginning (d0) and the last day of the experimental period (49 days). Blood samples were collected in Vacutainers® (BD, Franklin Lakes, NJ, USA) to measure blood cells. Bulls were fasted for 14 h before blood collection. Blood samples were obtained from the jugular vein. A total of 5 mL of blood was collected and mixed with anticoagulant (EDTA: diaminotetracticetilen acid and disodium salt). Hemogram (erythrocytes, hemoglobin, hematocrit, MCV - mean corpuscular volume, MHC - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration) and leukogram (eosinophils, segmented neutrophils, lymphocytes, and monocytes) measurements were performed according to Jain and Jain (1993). Samples were frozen until the analyses, during which they were centrifuged at 1,500 rpm for 15 minutes to collect the plasma.

All variables were tested for normality. Variables showing a normal distribution were analysed using the PROC GLM from SAS (Version 9.1.2.; SAS Institute Inc, Cary, NC, USA, 2004).

Data were compared using analysis of variance and the differences were tested by the Tukey test (5% probability). The following model was used for animal performance, feed efficiency, and total tract apparent digestibility:

$$Y_{ij} = \mu + D_i + e_{ij}$$

Where:

Y_{ij} = dependent variables.

μ = population average.

D_i = treatment (diet) effect.

e_{ij} = residual error.

For blood cell analyses, the effects of treatment and period (initial or final) were assessed with the following model:

$$Y_{ij} = \mu + D_i + P_j + Di^{*}Pj + e_{ij}$$

Where:

Y_{ij} = dependent variable.

μ = population average.

D_i = treatment (diet) effect.

P_j = collection period.

$D_i{}^{*}P_j$ = interaction between treatment and period.

e_{ij} = error.

When the effect was significant, differences between mean values were obtained by Tukey (5% probability).

Results

Propolis or essential oils did not affect ($p>0.05$) intake of DM (Kg/d or % of BW) and other nutrients (Table 3).

Final body weight (BW) and average daily gain (ADG) were greater ($p<0.05$) for bulls fed the OIL-based diet compared with bulls fed CON and PRO-based diets (Table 4). The DM conversion was 1.0 Kg greater ($p<0.05$) for bulls fed the OIL-based diet than the CON-based diet and 0.80 Kg greater for bulls fed the PRO-based diet. Likewise, crude protein conversion and DM efficiency were greater in the OIL diet ($p<0.01$ and $p<0.04$, respectively) compared to the other diets (CON or PRO).

Table 3. Feed intake (Kg/d and % body weight) of crossbred bulls finished in feedlot.

Item	Diets			SEM[4]	p>F
	CON[1]	PRO[2]	OIL[3]		
Dry matter, Kg/d	8.40	8.47	8.69	0.417	0.54
Dry matter, % BW	2.38	2.40	2.44	0.082	0.50
Organic matter, Kg/d	7.98	8.05	8.26	0.392	0.54
Crude protein, Kg/d	1.01	1.01	1.04	0.038	0.78
Ether extract, Kg/d	0.29	0.29	0.30	0.013	0.81
Neutral detergent fiber, Kg/d	2.64	2.70	2.78	0.136	0.78
Neutral detergent fiber, % BW	0.75	0.76	0.78	0.028	0.76
Acid detergent fiber, Kg/d	1.58	1.59	1.66	0.085	0.75
Total carbohydrates, Kg/d	6.68	6.75	6.91	0.294	0.86
Non fibrous carbohydrates, Kg/d	4.03	4.04	4.13	0.161	0.90
Total digestible nutrients, Kg/d	6.45	6.46	6.67	0.288	0.84

[1]Control diet. [2]Diet with propolis inclusion. [3]Diet with essential oils inclusion. [4]Standard error of means.

Table 4. Animal performance and feed efficiency of young crossbred bulls reared in feedlot.

Item	Diets			SEM[4]	p>F
	CON[1]	PRO[2]	OIL[3]		
Initial weight, Kg	320	320	320	8.711	0.99
Final weight, Kg	382^b	385^b	396^a	10.728	0.06
Average daily gain, Kg	1.26^b	1.32^b	1.53^a	0.101	0.05
Dry matter conversion	6.82^b	6.62^b	5.82^a	0.344	0.05
Crude protein conversion	0.82^b	0.79^b	0.70^a	0.041	0.01
Dry matter efficiency	0.15^b	0.15^b	0.17^a	0.009	0.04

[1]Control diet. [2]Diet with propolis inclusion. [3]Diet with essential oils inclusion. [4]Standard error of means. Means followed by different superscript letters indicate significant difference between rows (Tukey' test).

The addition of propolis and essential oils did not affect ($p>0.05$) the apparent digestibility of DM, organic matter, crude protein, ether extract, neutral detergent fiber, total carbohydrates, non-fibrous carbohydrates, and total digestible nutrients (Table 5).

Table 5. Apparent total digestibility of DM and other nutrients of young crossbred bulls finished in feedlot (g/Kg).

Nutrients	Diets			SEM[4]	p>F
	CON[1]	PRO[2]	OIL[3]		
Dry matter	661.2	690.4	671.3	40.081	0.27
Organic matter	616.8	648.1	629.9	18.352	0.31
Crude protein	548.6	594.2	585.4	24.603	0.16
Ether extract	724.7	774.4	753.8	38.788	0.33
Neutral detergent fiber	450.7	470.1	458.0	23.780	0.68
Acid detergent fiber	340.2	382.4	345.7	19.888	0.39
Total carbohydrates	640.1	667.6	648.3	18.382	0.38
Non fibrous carbohydrates	821.0	857.6	833.6	19.893	0.25
Total digestible nutrients	761.6	735.7	716.0	23.641	0.37

[1]Control diet. [2]Diet with propolis inclusion. [3]Diet with essential oils inclusion. [4]Standard error of means.

No interaction was observed ($p>0.05$) between diets vs. period for red and white blood cells. Thus, results are presented and discussed as principal effects.

Addition of propolis or essential oils did not affect ($p>0.05$) red blood cell means (erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentrations), platelets, white blood cell means (leukocytes, eosinophils, segmented neutrophils, lymphocytes, and monocytes), and plasma protein (Table 6) at initial and final collection.

Red blood cell concentrations in all treatments were higher ($p<0.05$) when comparing initial values and final ones, except for MCHC cells, which were similar (Table 6). However, white blood cell concentrations were lower at the 49th day of the experiment, except for lymphocyte cell concentrations, which were similar (Table 6). Likewise, plasma protein concentration decreased at the 49th day of the experiment (Table 6).

Table 6. Red and white blood cells, and plasma proteins of young crossbred bulls finished in feedlot.

Parameters	Diet						SEM[4]	Diet	Period	Period
	CON[1]		PRO[2]		OIL[3]					
	Initial	Final	Initial	Final	Initial	Final				
Red blood cells										
Erythrocytes, million/mm^3	8.80	9.84	8.34	9.70	8.50	9.48	0.379	0.32	<0.01	0.12
Hemoglobin, g/dL	13.0	15.4	12.6	15.0	12.4	15.0	0.543	0.41	<0.01	0.16
Hematocrit, %	37.2	44.4	36.6	43.4	35.7	43.2	1.497	0.33	<0.01	0.21
MCV[5], fL	42.5	45.6	44.2	44.9	42.4	46.0	1.457	0.45	0.05	0.11
MCH[6], pg	14.9	15.8	15.1	15.5	14.6	15.9	0.404	0.28	<0.01	0.24
MCHC[7], %	35.6	34.7	34.6	34.6	34.6	34.7	0.442	0.29	0.21	0.22
Platelets, million/m^3	330.4	226.1	355.9	199.3	400.2	234.2	6.041	0.33	<0.01	0.27
White blood cells										
Leukocytes, mm^3	17.85	12.53	17.54	12.48	19.13	11.89	1.431	0.33	<0.01	0.19
Eosinophils, mm^3	813.0	375.7	629.1	473.0	541.5	491.0	101.674	0.41	0.05	0.19
Segmented, mm^3	7.507	4.401	8.823	4.184	9.950	3.908	117.820	0.27	<0.01	0.24
Lymphocytes, mm^3	8.621	7.179	7.366	7.231	7.860	6.950	0.379	0.29	0.25	0.27
Monocytes, mm^3	908.2	580.8	721.3	595.2	777.9	547.7	10.670	0.35	0.01	0.29
Plasma proteins, mg/dL	7.05	6.94	7.10	6.87	7.19	6.95	0.110	0.52	0.05	0.16

Reference values: Erythrocytes (5-10 million/mm^3), Hemoglobin (8-15 g/dL), Hematocrit (24-46%), MCV — Mean corpuscular volume — (40-50 fL), MHC — Mean corpuscular hemoglobin — (11-17 pg); MCHC — mean corpuscular hemoglobin concentration (30-36%), Platelets (100-800 million/m^3), Leukocytes (4.000-12.000/mm^3), Eosinophlis (80-2.400/mm^3), Segmented (600-5.400/mm^3), Lymphocytes (1.800-9.000mm^3), Monocytes (80-840mm^3), Plasma proteins (5.7-8.0 mg/dL). [1]Control diet. [2]Diet with propolis inclusion. [3]Diet with essential oils inclusion. [4]Standard error of the mean. [5]Mean corpuscular volume. [6]Mean corpuscular hemoglobin. [7]Mean corpuscular hemoglobin concentration.

Discussion

As observed by Stelzer *et al.* (2009), the addition of propolis in the diet of dairy cows had no effect on feed intake. The feed intake observed in this study is similar to the values reported by Zawadzki *et al.* (2011) using growing and finishing cattle completed in feedlots on a diet with a forage-to-concentrate ratio of 48:52%. Likewise, Cruz *et al.* (2014) did not observe an effect of the addition of essential oils on feed intake of bulls finished in feedlots under similar conditions as in this experiment. DM feed intake for this animal category is between 2.2 and 2.5% BW (Maggioni *et al.*, 2009; Zawadzki *et al.*, 2011; Cruz *et al.*, 2014).

Mean crude protein intake for bulls on the three diets was 1.02 Kg/animal/d. Bulls with body weights between 320 and 360 Kg, depending on their genotype, feed, and environmental conditions, have a crude protein requirement of around 1.0 Kg/animal/d (NRC, 2000). The low intake of NDF and ADF was due to the low NDF and ADF content in glycerine. The mean TDN intake was 6.52 Kg/animal/d, which is the requirement of this category (NRC, 2000).

The mean final weight of bulls was 387 ± 10.7 Kg, and would be the ideal weight to initiate the final stage of finishing. Bulls that are on feedlots during the finishing phase reach body weights between 380 and 400 Kg (Dian *et al.*, 2009; Valero *et al.*, 2011; Zawadzki *et al.*, 2011).

Propolis has been used as a ruminal fermentation modulator (Prado *et al.*, 2010). Zawadzki *et al.* (2011) observed greater slaughter weights and average daily gains for Nellore bulls fed a diet containing alcoholic propolis extract. They attributed the improved performance observed to the antimicrobial properties of propolis. Furthermore, dietary inclusion of flavonoids can improve animal production, according to Benchaar *et al.* (2008).

Feed efficiency was better for bulls fed the OIL-based diet. Zawadzki *et al.* (2011) observed better DM efficiency for Nellore bulls fed a propolis extract-based diet using 52% roughage and 48% concentrate. According to the authors, the better DM efficiency may be due to the bioactive compounds in propolis, particularly the antibacterial action associated with presence of flavonoids in propolis extract. However, Albertí *et al.* (2005) and Devant *et al.* (2007) found no positive effect on feed efficiency of feedlot cattle by the addition of plant extracts.

Prado *et al.* (2010) observed lower DM digestibility when two different propolis extracts were included in the diets of cattle fed 72.5% roughage (sorghum silage and grass hay) and 27.5% concentrate. As observed in the present experiment, Ítavo *et al.* (2011) reported apparent DM digestibility close to 63% in sheep fed a 50% roughage (*Brachiaria brizhanta* grass hay) and 50% concentrate mix with four different levels of propolis extracts. The apparent total tract digestibility of crude protein was low (close to 576 g/Kg) compared with other studies. Previous studies reported that the addition of propolis extracts to the diet reduced the apparent digestibility of crude protein from 65.4 to 58.0% in cattle fed a mixture of 72.5% roughage and 27.5% concentrate (Prado *et al.*, 2010). Ítavo *et al.* (2011) observed apparent total tract digestibility of crude protein of about 75% in diets containing four levels of crude propolis extract. Thus, the method of preparation of propolis extract (crude residue, crude extract with alcohol extraction, and purified propolis extract with alcohol extraction) has a different effect on the apparent total tract digestibility of crude protein in several animal species, such as bovine and ovine. Prado *et al.* (2010) observed a reduction in the apparent total tract digestibility of ether extract in diets added with different propolis extracts fed to cattle. According to the authors, the likely reduction in apparent digestibility of ether extract was due to substances present in these products, which may have hindered the action of lipolytic bacteria. In contrast, Ítavo *et al.* (2011) observed no effect on the apparent digestibility of ether extract (65.0%) due to the addition of propolis in the diet sheep fed 50% forage and 50% concentrate; thus, the action of propolis varies according to its chemical composition, which varies with the flora of the region and the method for obtaining the propolis extract as well as with the bee species. The apparent digestibility of neutral detergent fiber (459 g/Kg) and acid detergent fiber (356 g/Kg) was lower than the digestibility of diets with different levels of forage and concentrate (Prado *et al.*, 2010; Ítavo *et al.*, 2011). Glycerine inclusion may have altered fiber digestibility of the diet. Ramos and Kerley (2012) observed a significant reduction in digestibility of diet components in beef cattle with the addition of 20% glycerine. No influence was observed in the composition of total digestible nutrients due to the additives. The value of total digestible nutrients obtained from the control diet was close to the present values for growing cattle (NRC, 2000), and the addition of propolis and essential oils did not reduce the levels of total digestible nutrients in the diet.

Hematological evaluation in cattle is used to assess disease in an animal, to evaluate groups of animals within a herd, to detect hidden diseases, and to guide clinical decisions. The red blood cell series are a reflection of the health and nutrition of animals (Jones and Allison, 2007). The values of the variables observed in this study for red blood cells are in accordance with the reference values described by Biondo *et al.* (1998). Furthermore, according to the values reported by Biondo *et al.* (1998) and Jones and Allison (2007), MCV, MCH, and MCHC values in our study are within the normal ranges for cattle.

Measuring of white blood cells in this study was aimed to monitor the health of bulls and observe the behaviour of these cells upon supplementation with propolis and essential oils, due to its recent introduction into ruminant nutrition. The results found in all groups are in accordance with the reference values for cattle during this phase of growth (Jones and Allison, 2007).

Plasma proteins increase or decrease in the plasma as a result of injury or inflammation. Plasma proteins in blood are investigated as a mean of detecting and monitoring inflammatory processes in ruminants (Jones and Allison, 2007). In summary, plasma proteins can provide valuable information to the clinician. Evaluation of plasma proteins is an essential adjunct to detect significant abnormalities in cattle (González *et al.*, 2000). We did not observe any inflammatory processes in the bulls during the experimental period. The results of this experiment are probably due to improved nutrition throughout the experimental period. At the beginning of the experimental (feedlot) period, all animals were treated for endo- and ecto-parasites that are common in the region. Moreover, these animals had no further contact with other animals during the experimental period and were kept on concrete floor stalls that were washed three times a week. The correct management and nutrition during the feedlot period could explain the low levels of white blood cells and plasma proteins in our experiment.

In conclusion, the addition of propolis to the diet of bulls finished in feedlots did not change the average daily weight gain, feed efficiency or apparent digestibility. Therefore, the addition of propolis to the diet of cattle in feedlots is not justified. However, the addition of essential oils to the diet of bulls improved both the average daily weight gain and feed efficiency. Thus, essential oils can be added to the diets of cattle to improve performance and feed efficiency, while acting as a replacement for other products such as antibiotics and ionophores.

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Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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