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Effect of essential and vegetable oil blend supplementation on animal performance, feed intake, rumen fermentation and rumen microbial populations of crossbred steers finished in a pasture system

Efeito da suplementação com uma mistura de óleos essenciais e vegetais sobre o desempenho animal, consumo de ração, fermentação ruminal e populações microbianas ruminais de novilhos mestiços terminados a pasto

Efecto de la suplementación con mezclas de aceites esenciales y vegetales sobre el rendimiento animal, consumo de alimentos, fermentación ruminal y poblaciones microbianas del rumen en novillos cruzados terminados a pasto

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Abstract

Recent years have seen a general increase in consumer concern regarding the profile of additives in animal feed and food sources, prompting the industry to study essential oil blends that have been promoted to replace synthetic products. This study evaluated the effect of essential oil blends supplementation on animal performance, feed intake, rumen fermentation and rumen microbial populations in crossbred steers finished in a pasture system. A total of 40 steers (½ Bons Mara vs. ½ Nellore) with a mean age of 20 ± 2.0 months and a mean body weight of 416.9 ± 5.56 kg were distributed in a completely randomized design with five diets and eight replications per diet. The five experimental diets were: CONT – basal diet, and oil blend inclusion in dosages of 1500, 3000, 4500 or 6000 mg/animal/d. Animal performance was similar among diets. The forage intake, crude protein, neutral detergent fiber, ethereal extract, non-fibrous carbohydrates, and the digestibility of crude protein, neutral detergent

fiber, and non-fibrous carbohydrates were greater when essential oil blends were fed to steers. There was an increase in the concentrations of rumen ammonia nitrogen, and propionic and isovaleric volatile fatty acids when essential oil blends were used. The microbiological population of the rumen was similar among the five diets. Our results suggest that the inclusion of doses above 1500 mg/animal/ is high for livestock grazing on temperate pasture, further studies are needed to identify a promising dose to replace antimicrobial additives.

Keywords: Cashew oil; Castor oil; Cattle; Clove oil; Natural plant extract.

Resumo

Nos últimos anos houve um aumento geral na preocupação dos consumidores em relação ao perfil de aditivos na alimentação animal e nas fontes alimentares, levando a indústria a estudar misturas de óleos essenciais que foram promovidos para substituir produtos sintéticos. Este estudo avaliou o efeito da suplementação de misturas de óleos essenciais e vegetais sobre o desempenho animal, consumo de ração, fermentação ruminal e populações microbianas ruminais em novilhos mestiços terminados a pasto. Foram utilizados 40 novilhos (½ Bons Mara vs. ½ Nelore) com idade média de 20 ± 2,0 meses e peso corporal médio de 416,9 ± 5,56 kg, distribuídos em um delineamento inteiramente casualizado com cinco dietas e oito repetições por dieta. As cinco dietas experimentais foram: CONT - dieta basal e inclusão de mistura de óleos nas dosagens de 1500, 3000, 4500 ou 6000 mg/animal/d. O desempenho dos animais foram semelhante entre as dietas. O consumo de forragem, proteína bruta, fibra em detergente neutro, extrato etéreo, carboidratos não fibrosos e a digestibilidade da proteína bruta, fibra em detergente neutro e carboidratos não fibrosos foram maiores quando misturas de óleos essenciais foram fornecidas aos novilhos. Houve aumento nas concentrações de nitrogênio amoniacal ruminal e ácidos graxos voláteis propiônicos e isovaléricos quando utilizadas as misturas de óleos essenciais. A população microbiológica do rúmen foi semelhante entre as cinco dietas. Nossos resultados sugerem que a inclusão de doses acima de 1500 mg/animal/d é elevada para rebanhos em pastagem temperada, sendo necessários mais estudos para identificar uma dose promissora para substituir os aditivos antimicrobianos.

Palavras-chave: Óleo de caju; Óleo de mamona; Bovinos; Óleo de cravo; Extrato natural de plantas.

Resumen

En los últimos años está habiendo un incremento general por parte del consumidor sobre la preocupación por los aditivos y las fuentes alimenticias usadas en la alimentación animal.

Incentivando a la industria a estudiar los aceites esenciales, los cuales han ido reemplazando los productos sintéticos. Este estudio evaluó el efecto de la suplementación de una mezcla de aceites esenciales en el rendimiento animal, ingesta, fermentación ruminal y la población ruminal en novillos mestizos finalizados en un sistema de pastoreo. Un total de 40 novillos (½ Bons Mara vs. ½ Nellore) con una edad promedio de 20 ± 2.0 meses y un peso promedio de 416.9 ± 5.56 kg fueron distribuidos en un diseño completamente al azar con cinco dietas y ocho replicas por tratamiento. Las cinco dietas experimentales fueron: CONT – dieta control; y la inclusión de una mezcla de aceites en una dosis de 1500, 3000, 45000 y 6000 mg/animal/d. El rendimiento animal fue similar en todos los tratamientos. La ingesta de forraje, proteína cruda, fibra detergente neutro, extracto etéreo, carbohidratos no fibrosos y la digestibilidad de la proteína cruda, fibra en detergente neutro y de los carbohidratos no fibrosos fueron mejor cuando se suministró la mezcla de los aceites en la alimentación de los novillos. Hubo un incremento en la concentración de nitrógeno amoniacal en el rumen y de los ácidos grasos volátiles propiónico e isovalenico cuando fueron usadas las mezclas de aceites. No hubo alteración en la población microbiológica del rumen. Los resultados sugieren que la inclusión de la dosis de 1500 mg/animal/d en bovino terminados a pasto, pueden resultar una alternativa promisoria para remplazar aditivos antimicrobiales sintéticos.
Palabras clave: Aceite de marañon; Aceite de rícino; Bovinos; Aceite de clavo; Extracto natural de plantas.

1. Introduction

Recent years have seen a general increase in consumer concern regarding the profile of additives in animal feed and food sources, prompting the industry to study essential oil blends (EOB) have been promoted to replace synthetic products (Monteschio et al., 2020; Monteschio et al., 2017; Rivaroli et al., 2016; Valero et al., 2014; Valero et al., 2016).

Among the wide variety of EOB currently available, vegetable and essential oils are the most commonly used as modulators of microbial flora (Monteschio et al., 2017; Ornaghi et al., 2017; Rivaroli et al., 2016). The essential oil of clove (*Eugenia caryophyllus*) has shown to have a positive effect on rumen modulation in vitro (Remmal et al., 2011) as well as on animal performance and carcass characteristics percentage (Monteschio et al., 2017; Ornaghi et al., 2017; Rivaroli et al., 2017). Alternative vegetable oils also have a proven antimicrobial capacity, in addition to their use as energy supply, including castor oil (*Ricinus communis* L.) and cashew oil (*Anacardium occidentale*) (Cruz et al., 2014; Prado et al., 2016;

Valero et al., 2014) Essential oils may be microencapsulated in either their natural form or as similar synthetic molecules. Such microencapsulated additives are used to preserve the oil molecules, which are volatiles (Monteschio et al., 2017; Rivaroli et al., 2017).

Previous studies on crossbred beef cattle finished in feedlots have shown that various natural compounds may improve animal performance and favorably alter rumen metabolism (Ornaghi et al., 2017; Rivaroli et al., 2017; Valero et al., 2014). However, similar studies focusing on semi-intensive or pasture systems remain scarce.

The hypothesis of this work was that of microencapsulated principle blend (eugenol, thymol and vanillin) added in the diets of in crossbred steers finished in a pasture system could improve animal performance. Thus, this study was realized to evaluated the effect of essential oil blends supplementation on animal performance, feed intake, rumen fermentation and rumen microbial populations in crossbred steers finished in a pasture system.

2. Methodology

This study was conducted in strict conformity with the Brazilian legislation on experimentation involving the use of animals adopted by the National Council of Experimental Control (CONCEA) and was approved by the Ethics Committee in Animal Use (CEUA) of the State University of Maringá, located in Maringá, Paraná, south Brazil, under approval number 9827130218. This work is a quantitative research (Pereira et al., 2018).

2.1. Location, animals and diets

The experiment was conducted at a rural property located in Campina da Lagoa (24°35'34.4" S52°36'38.3"W), Paraná, South Brazil, from July to October in 2018. This study period was selected as it encompassed the regional dry-to-rainy transition season, thus making it possible to employ temperate pastures due to the lower temperatures, as well as adopt the local cultural practice used for the deposition of organic matter in the soil in the soybean off-season. The average rainfall was 33 mm in July, 201 mm in August, 49 mm in September, and 58 mm in October. The average availability of forage dry matter (DM) during the experiment was 4489.6 kg/ha.

A total of 40 steers (½ Bons Mara vs. ½ Nellore) with a mean age of 20 ± 2.0 months and a mean body weight of 416.9 ± 5.56 kg were kept in a pasture of white oat (*Avena sativa*) consortium with ryegrass (*Lolium perene*), covering an area of 70 ha with continuous

intensive stocking. The steers had free access to water through water fountains located in each paddock.

The experimental design was completely randomized, with five diets eight replications per diet. The five experimental diets were: CONT – basal diet; OB15 - basal diet and 1500 mg/animal/d of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated; OB30 - 3000 mg/animal/d of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated; OB45 - 4500 mg/animal/d of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated; OB60 - 6000 mg/animal/d of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (Table 1). These concentrations represent typical amounts of compounds from plant extracts and EO supplied to ruminants' diets (Ornaghi et al., 2020, 2017; Rivaroli et al., 2020; Souza et al., 2019).

Table 1. Doses of the oil blend supplemented in the experimental diets.

Oil blend	Experimental diet				
	CONT[1]	OB15[2]	OB30[3]	OB45[4]	OB60[5]
Clove essential oil[6]	0	500	1000	1500	2000
Cashew oil[7]	0	250	500	750	1000
Castor oil[7]	0	250	500	750	1000
Eugenol/thymol/vanillin microencapsulated[7]	0	500	1000	1500	2000
Total, mg/animal/day	0	1500	3000	4500	6000

[1]CONT – basal diet.
[2]OB15 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (1500 mg/animal/d).
[3]OB30 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (3000 mg/animal/d).
[4]OB45 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (4500 mg/animal/d).
[5]OB60 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (6000 mg/animal/d).
Source: Authors.

The animals were sent daily to the corral, where the concentrate from each treatment was provided once daily (0900 h) in individual pens (with latches) in the amount of 1.77 kg DM animal-1 (composition g/kg, as fed: 1672.7 g cracked corn, 13.3 g soybean meal, 46 g mineral salt, 34.3 g limestone, 11.7 g dicalcium phosphate, and 4 g yeast), with only the amount of additives changed according to the dosages displayed in Table 2.

Table 2. Ingredients and chemical composition of diets.

Ingredients	Chemical composition							
	DM[1]	CP[2]	OM[3]	EE[4]	NDF[5]	ADF[6]	ME[7*]	Diet, %
Forage, % DM	-	-	-	-	-	-	-	-
Oat + ryegrass	22.8	11.2	67.1	1.8	66.4	39.6	250.9	-
Concentrate, % DM	-	-	-	-	-	-	-	-
Cracked corn	88.9	10.0	99.1	3.5	17.7	4.4	325.38	94.5
Soybean meal	88.6	49.7	93.7	1.3	13.7	5.9	260.3	0.75
Salt	98.0	-	-	-	-	-	-	2.5
Limestone	98.0	-	-	-	-	-	-	1.94
Dicalcium phosphate	98.0	-	-	-	-	-	-	0.65
Yeast[8]	98.0	30.0	98.0	-	-	-	-	-
Diet, %	89.1	19.6	94.6	2.83	16.1	4.65	298.6	-

*Values expressed in Mcal/kg DM.
[1]DM dry matter.
[2]CP crude protein.
[3]OM organic matter.
[4]EE ether extract.
[5]NDF neutral detergent fiber.
[6]ADF acid detergent fiber.
[7]ME metabolizable energy.
[8]BIOSAF®, *Saccharomyces cerevisiae* from strain Sc 47, at a concentration of 1×10^{10} cfu/g of product.
Source: Authors.

The clove essential oil contained 845 g/kg, 133 g/kg, and 13 g/kg of eugenol, carophylene, and eugenyl acetate, respectively (Biondo et al., 2017); the cashew oil contained 750 g/kg anarcardic acid, 153 g/kg cardol, and 41 g/kg cardanol; and the castor oil contained 895 g/kg ricinoleic acid, 42 g/kg linoleic acid, and 30 g/kg oleic acid. Clove essential oils were obtained from Ferquima® (Vargem Grande Paulista, São Paulo, Brazil). The cashew oil, castor oil, and microencapsulated blend (eugenol, thymol, and vanillin active principles) were obtained from Safeeds® (Cascavel, Paraná, Brazil). The liquid textured oils were first added one at a time until completely homogenized, with the microencapsulated oils added later with the concentrate in a commercial mixer every two weeks, when the diets were prepared. Hence, the inclusion of EOB was calculated, adjusting the inclusion according to the intake of dry matter/day per animal, to maintain a constant dosage per animal/day. The control treatment was done first to avoid contamination.

2.2. Experimental procedure and sampling

Animals were adapted to the experimental procedures and management system for 14 days before the beginning of the experimental period. The pasture was evaluated at pre-established 20-day intervals, totaling 80 days of the trial period. For performance evaluation, the animals were weighed on a trunk balance (Toledo® MGR 3000 JUNIOR) at the beginning and end of the experiment after 14 h fasting.

Samples used for the chemical composition analysis of the pasture consumed by the animals were obtained by hand plucking every 20 days to quantify the forage mass, making a cut approximately 1 cm above the ground in ten randomly chosen areas delimited by a metal square (0.5 m^2).

To evaluate voluntary intake and digestibility, a 12-day digestibility trial was carried out from the 40th day of the experimental period. Estimation of fecal excretion was undertaken by feeding the animals titanium dioxide as an external marker (Detmann et al., 2012) supplied as a supplement at 10 g/animal/d (Titgemeyer et al., 2001). Forage dry matter intake (DMI) was estimated by using indigestible neutral detergent fiber as an internal marker (Detmann et al., 2012; Zeoula et al., 2002).

The first seven days of the experiment were used to stabilize marker flow in the gastrointestinal tract, while the last 5 days were used for feces collection at different times (at 06:00, 09:00, 12:00, 15:00 and 18:00 hours, respectively). The steers were led to the trunk and fecal samples of approximately 200 g were collected directly from the rectum and stored in a cold chamber at -26ºC. Samples were then oven-dried (60° C/72 h) and proportionally pooled per animal. On the 7th day of the digestibility assay, a forage sample was obtained via the hand-plucking method to estimate voluntary intake and digestibility.

Samples of ruminal fluid were collected via oral stomach tube (11 mm diameter) and manual vacuum aspirator (TE-058, Tecnal in Piracicaba, São Paulo, Brazil), filtered through a double cotton cloth and conditioned according to the analysis to be used. A total of 400 mL ruminal fluid was sampled from several different anatomical regions of the rumen.

The steers were slaughtered at approximately 23 months of age, at which time their average body weight was 494.1 kg, in a commercial slaughterhouse (Campo Mourão, Paraná, Brazil) following the slaughtering standards of the State Inspection Service Brazilian Legislation.

2.3. Sample processing

The samples used for quantifying the chemical composition of the ingredient's diets, forage, and feces were ground in a knife mill with a 2-mm sieve. The DM content was determined by oven-drying at 65°C for 24 h and then drying at 135°C for 3 h (Method 930.15) (AOAC, 2005). The organic matter (OM) content was calculated as the difference between the DM and ash contents, with ash determined by combustion at 550° C for 5 h (method 930.05) (AOAC, 2005). The N content in the samples was determined by the Kjeldahl for crude protein (CP) (method 976.05) (AOAC, 2005). The ether extract (EE) by Soxhlet method (method 920.39) (AOAC, 2005). For analysis of neutral detergent fiber (NDF) and acid detergent fiber (ADF), samples were treated with α-thermostable amylase without sodium sulfite and corrected for ash residue (Mertens, 2002) and residual nitrogen compounds (Licitra et al., 1996).

Indigestible neutral detergent fiber (iNDF) was analyzed as described by Valente, 2011. Sample amounts of 1.5 g were added to pre-weighed polyester cloth Saatifil PES 12/6 (Saatitech S.p.A., 22070 in Veniano, Como, Italy) with a pore size of 12 μm and open surface area of 6%. The bags were incubated for 288 h in the rumen of 2 steers fed a diet consisting of 50% corn silage and 50% concentrate (DM basis) at maintenance level (Huhtanen et al., 1994). After removal from the rumen, the bags were rinsed, dried at 45° C for 48 h, and weighed. Residues were then analyzed for NDF in an Ankom 200/220 Fiber Analyzer (Ankom Technology Corp in USA). Heat-stable α-amylase (Mertens, 2002) was used in the determination of NDF.

Non-fiber carbohydrates (NFC) were calculated according to Detmann et al. (2012). For converting metabolizable energy (ME) requirement into digestible energy requirements, the factor of 0.82 was used.

Fecal samples were evaluated for titanium dioxide content via both atomic absorption spectrophotometry (Thermo Scientific, Genesys Scanning 10 mV in USA) (Detmann et al., 2012; Williams et al., 1962) colorimetric methods (Titgemeyer et al., 2001). Fecal excretion and forage DMI were estimated by rationing the quantity of TiO2 offered and calculating the concentration in feces.

Ruminal pH was estimated using a digital potentiometer (Hanna HI 2211 in Limena, Italy). The method described by Detmann et al. (2012) was used for the analysis of ammoniacal nitrogen concentrations. Short-chain fatty acid and gas quantification was conducted via gas chromatography using an SP-2560 capillary column (100 m × 0.25 mm in

diameter 0.02 mm thick) (Palmquist & Conrad, 1971). The concentration of volatile fatty acids was evaluated by gas chromatography (GC-2010 Plus chromatograph, Shimadzu, Barueri, Brazil) equipped with an AOC-20i auto-sampler, Stabilwax-DA™ capillary column (30 m, 0.25 mm ID, 0.25 μm df; Restek©) and a flame ionization detector according to Del Valle et al. (2018).

Macroscopic analyzes of color (1 – olive green, 2 – brownish-green, 3 – yellowish-brown color, 4 – grey and 5 – darker greenish), odor (1 – aromatic, 2 – acid and 3 – putrid) and viscosity (1 – viscous, 2 – viscous or frothy bloat and 3 – lightly viscous) were performed according to Feitosa (2014) and the physical-chemical analyzes of potential redox (1 – active (0 to 3 min); 2 – normal (3 to 5 min) and 3 – reduced (greater than 5 min), sedimentation and flotation time (1 – active (0 to 4 min), 2 – normal (4 to 8 min) and 3 – reduced (greater than 8 min) and density and quantification of protozoa (1 – absent, 2 – little, 3 – normal and 4 – abundant) according to Dehority (1984).

2.4. Statistical analyses

Each animal was considered an experimental unit. All studied variables were tested for normality, with those exhibiting a normal distribution submitted to variance analysis (ANOVA) via an adjusted regression model (animal performance, feed intake, digestibility, ruminal pH, concentration of ruminal ammoniacal nitrogen, concentration of volatile fatty acids, and microbiological protozoa viability), and those that did not subjected to the Kruskal-Wallis non-parametric method (all ruminal fluid parameters except for microbiological protozoa viability). Orthogonal contrast was used to evaluate the effects of the control treatment versus oil blend. In all statistical analyses, the diet was considered a fixed effect. Differences between means were compared using the Tukey test ($P < 0.05$). The statistical program used was the SPSS v.21 (IBM Corporate Headquarters in Armonk, NY).

3. Results and Discussion

The chemical compositions of the forage and concentrate are shown in Table 2. Animals had restricted access to the concentrate containing the EOB (1.77 kg DM/day), and *ad libitum* access to forage.

An average CP value of 11.2% was recorded for the oat and ryegrass consortium. This value is somewhat lower than those of above 15% found by Roso et al. (2000) and Rocha et al. (2007), whose mean value above 15%, but similar to the 10.1% reported by

Prohmann et al. (2004). It should be noted that in the present study, grazing began near the end of the ryegrass vegetative cycle. This consortium is widely used in southern Brazil since oats make it possible to anticipate the use of pasture, and ryegrass prolongs this cycle.

The average NDF value was 66.0% for the pasture, with average ADF 39.6%, which may limit intake. Mean values of DM, OM, EE, and ME were 22.8%, 67.1%, 1.8%, and 250.9 Mcal/kg, respectively, all of which are somewhat below levels normally found. However, in addition to the later plant stage, frosts were also recorded throughout the experiment (Prohmann et al., 2004; Rocha et al., 2007).

Although the addition of EOB did not influence the final live weight (FBW) of the steers, it did result in a linear decrease ($P < 0.07$) in the average daily gain (ADG) and consequently also the total average gain (Table 3). Nevertheless, such effects were not evident in steer performance, and can thus be explained by the decrease in forage intake (NA30, NA45, and NA60), CP intake, and fiber digestibility (NA15, NA30, and NA45).

Table 3. Animal performance, feed intake and *in vivo* digestibility of steers with oil blend in the diet.

Items	Experimental diet					SEM[6]	*P -value*		
	CON[1]	OB15[2]	OB30[3]	OB45[4]	OB60[5]		L	Q	0 *vs* OB
Performance									
Initial weight	410.8	411.0	410.3	411.9	411.4	6.96	0.966	0.999	0.723
Final weight	494.3	485.3	477.4	482.5	476.0	7.32	0.453	0.726	0.158
Average daily gain	1.06	0.94	0.85	0.89	0.82	0.04	0.068	0.156	0.831
Intake, kg/d									
Dry matter	10.64	11.31	9.61	9.68	9.40	0.186	0.002	0.011[a]	0.494
Dry matter forage	8.88	9.55	7.85	7.92	7.64	0.186	0.002	0.011[b]	0.949
Crude protein	1.24	1.31	1.13	1.13	1.10	0.209	0.002	0.009[c]	0.586
Neutral detergent fiber	6.74	7.18	6.05	6.11	5.92	0.124	0.002	0.011[d]	0.493
Ether extract	0.39	0.42	0.36	0.39	0.35	0.006	0.002	0.011[e]	0.504
Non fibrous carbohydrate	1.88	2.01	1.67	1.69	1.63	0.03	0.002	0.010[f]	0.517
Apparent digestibility, g/kg DM									
Dry matter	581.1	589.8	585.6	542.3	593.5	0.655	0.785	0.788	0.822
Crude protein	843.3	624.8	583.2	519.0	583.7	2.829	0.001	0.001[g]	0.001
Neutral detergent fiber	590.8	581.9	572.3	580.2	611.8	0.488	0.305	0.068	0.524
Ether extract	761.6	814.2	802.8	766.4	783.9	0.659	0.969	0.224	0.110
Non fibrous carbohydrate	331.6	563.7	641.5	418.0	534.8	3.066	0.235	0.050	0.017

[1]CONT – basal diet.
[2]OB15 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (1500 mg/animal/d).
[3]OB30 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (3000 mg/animal/d).
[4]OB45 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (4500 mg/animal/d).
[5]OB60 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (6000 mg/animal/d).
[6]Standard error of means.
[a]$\hat{Y}=10,69+0.15X-0.76X^2$ ($r^2=0.483$); [c]$\hat{Y}=9.40-0.19X-0.02X^2$ ($r^2=0.359$); [d]$\hat{Y}=1.31-0.03X-0.001X^2$ ($r^2=0.444$); [e]$\hat{Y}=7.16-0.22X-0.007X^2$ ($r^2=0.430$); [f]$\hat{Y}=0.20-0.007X$ ($r^2=0.432$); [g]$\hat{Y}=2.01-0.06X-0.002X^2$ ($r^2=0.437$).
Source: Authors.

A non-significant linear decrease in ADG was recorded as the level of OB in the diet increased (P = 0.07). In addition, feed intake exhibited a quadratic reduction (P < 0.05) in all variables (DM, CP, NDF, EE, and NFC). These findings are important, as the literature is very scarce regarding the effect of OB or their components on the feed intake and performance of ruminants, especially those in the pasture.

As the rumen is the anaerobic chamber in which DM and food fiber are digested, changes in the digestibility of these components are important indices used in the evaluation of NA impact on ruminant digestion. In the present study, whereas no differences were observed in the digestibility of DM (P > 0.05), a quadratic effect was recorded for NDF digestibility (P < 0.05). These results agree with those of Metwally et al. (2016) for Friesian dairy cows fistulated with the addition of a 1 g/d blend of various essential oils, including thymol, m-cresol, guaiacol, eugenol, and resorcinol.

Animal performance was found to be directly dependent on daily feed intake (Maggioni et al., 2009), with a quadratic effect recorded on the digestibility of nutrient CP and NDF (P < 0.05). Orthogonal contrast analysis also revealed variation in CP digestibility between treatments with and without oil blend (P < 0.05).

The effect of the selected additives on forage consumption and fiber digestibility varied with dose, with the highest intake of DM observed in treatment NA15, and the lowest intake in treatment NA60. This increase in DMI also influenced the intake of other nutrients (CP, NDF, EE, and NFC). Several feedlot studies have shown that high doses of OB may inhibit the growth of certain cellulolytic ruminal bacteria, which may compromise fiber digestion and limit consumption due to an increased rumen filling effect (Maggioni et al., 2009). The results found here are similar to those reported by McIntosh et al. (2003), who fed fistulated Holstein-Friesian cows with a 1 g/d mix of thymol, eugenol, vanillin, and limonene essential oils, and Lin et al. (2013), who fitted Hu sheep with ruminal and duodenal fistula to

investigate the effects of a 1 g/d mixture of essential oils of clove, oregano, cinnamon and lemon (using 0.5 or 1.0 g/d combinations of the active components eugenol, carvacrol, citral, and cinnamaldehyde).

A lower population of cellulolytic bacteria may lead to a reduction in fiber degradation, reducing the access of proteolytic bacteria to the nitrogen bound to the fibrous fraction, and indirectly reducing protein degradation (Ríspoli et al., 2009).

The current results suggest that doses above 1500 mg/animal/d are too high for cattle grazing in temperate grassland, and thus studies involving doses below this value are required. Nevertheless, higher NFC digestibility was observed in treatments that received oil blend in the diet.

The mean ruminal pH of 7.74 was unaffected by the addition of EOB at the levels used in the present study (Table 4). Although this value is higher than that reported elsewhere for cattle, ruminal pH can be influenced by the fluid collection method employed, which frequently varies between studies (Salles et al., 2003).

Table 4. Ruminal pH, concentration of ruminal ammoniacal nitrogen and concentration of volatile fatty acids (VFA) of steers with oil blend in the diet.

Items	Experimental diet					SEM[6]	*P-value*		
	CON[1]	OB15[2]	OB30[3]	OB45[4]	OB60[5]		L	Q	0 *vs* OB
pH	7.76	7.79	7.73	7.63	7.82	0.034	0.880	0.656	0.891
Ammonia nitrogen, mg/dL	3.72	6.2	17.75	13.93	10.81	2.972	0.018	0.001[a]	0.035
VFA concentration mmol/dL									
Total	43.76	43.98	53.60	57.88	49.86	1.572	0.415	0.714	0.339
Acetic	32.04	29.62	35.59	35.02	33.74	1.992	0.691	0.925	0.170
Propionic	6.16	4.49	6.82	7.56	6.30	0.501	0.549	0.832	0.056
Isobutyric	0.59	0.55	0.63	0.87	0.54	0.042	0.580	0.232	0.206
Butiryc	6.40	5.15	7.43	6.45	4.71	0.440	0.969	0.949	0.218
Isovaleric	0.92	0.82	1.19	1.31	0.99	0.073	0.271	0.142	0.053
Valeric	0.39	0.35	0.42	0.49	0.39	0.033	0.594	0.801	0.256

[1]CONT – basal diet.
[2]OB15 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (1500 mg/animal/d).
[3]OB30 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (3000 mg/animal/d).
[4]OB45 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (4500 mg/animal/d).

[5]OB60 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (6000 mg/animal/d).
[6]Standard error of means.
[a]$\hat{Y} = -11.44+15.24X-2.15X^2$ ($r^2=0.808$).
Source: Authors.

RAN concentrations exhibited both quadratic behavior ($P < 0.05$) and an orthogonal contrast effect ($P < 0.05$). The higher values observed here are potentially linked to lower NFC fermentation (Table 3) since the synthesis of microbial protein in the rumen is dependent on carbohydrate availability.

Metwally et al. (2016) found a strong increasing tendency in the degradability of crude protein in protein-rich foods such as soybean and canola meal, possibly reflecting the activation of proteolytic bacteria due to the addition of EOB. In contrast, McIntosh et al. (2003) and Newbold et al. (2004) observed a reduction in the ammoniacal nitrogen production rate in cows and sheep fed respectively with a 1 g and 100 mg/d mix of thymol, eugenol, vanillin, and limonene essential oils, suggesting that these additives inhibited the activity of ammonia-producing bacteria.

The total concentration of VFA was also similar between treatments, as found by other authors (Metwally et al., 2016). However, when comparing the control treatment with NA addition, higher production of propionic and isovaleric acids was observed in the latter (P = 0.05). Ruminal concentrations of propionic acid indicate fermentation of soluble sugars and starch, while higher concentrations of isovaleric acid are indicative of the fermentation of amino acids, suggesting a modification of the microbial population in the rumen. However, Busquet 2006, who examined different doses of 12 plant extracts and 6 secondary plant metabolites, found that some oils affected rumen fermentation, with total VFAs reduced with a linear increase in the molar concentration of propionate.

Movement of the rumen-reticulum promotes rumination (Elischer et al., 2013). In the present study, animals in treatment NA30 exhibited a greater number of ruminal movements ($P > 0.05$) (Table 5), as well as lower NDF digestibility. In contrast, treatment NA60 was associated with a lower number of ruminal movements and higher NDF digestibility.

Table 5. Ruminal fluid parameters of steers with oil blend in the diet.

Ruminal fluid parameters	Experimental diet					SEM[6]	*P -value*	0 *vs* OB
	CON[1]	OB15[2]	OB30[3]	OB45[4]	OB60[5]			
Macroscopic								
Ruminal movements	2.2[ab]	2.2[ab]	2.4[a]	1.8[ab]	1.6[b]	0.122	0.042	0.595
Color[7]	2.4	2.6	2.8	2.6	2.6	0.153	0.479	0.592
Odor[8]	1.0	1.0	1.0	1.0	1.0	0.001	0.999	0.999
Consistency[9]	1.8[ab]	1.2[b]	1.0[b]	1.0[b]	2.4[a]	0.165	0.002	0.911
Sedimentation and flotation[10]	1.4[b]	2.0[a]	1.8[a]	1.2[b]	1.6[ab]	0.115	0.002	0.453
Redox potential[11]	1.6[a]	1.8[a]	1.0[b]	1.2[ab]	1.2[ab]	0.114	0.035	0.452
Microbiological protozoa								
Total count, x10³/mL	212.9	210.0	276.9	287.5	223.5	29.011	0.640	0.601
Viable, %	66	72	76	80	84	3.830	0.106	0.402
Density[12]	1.8	2	1.8	1.2	1.6	0.138	0.074	0.750
Great[13]	2.0	1.8	1.0	1.8	1.6	0.190	0.092	0.456
Medium[14]	2.8	2.8	2.8	3	3.2	0.140	0.355	0.915
Small[15]	3.0	3.4	3.0	3.0	3.0	0.099	0.214	0.480

[a,b,c] Means within rows with different superscripts differ (P < 0.05).
[1]CONT – basal diet.
[2]OB15 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (1500 mg/animal/d).
[3]OB30 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (3000 mg/animal/d).
[4]OB45 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (4500 mg/animal/d).
[5]OB60 - basal diet and blend of clove essential oil, cashew oil, castor oil, and eugenol/thymol/vanillin microencapsulated (6000 mg/animal/d).
[6]Standard error of means.
[7]Color (1 - olive green, 2 - brownish green, 3 - yellowish brown color, 4 - grey and 5 - darker greenish). [8]Odor (1 - aromatic, 2 - acid and 3 - putrid).
[9]Consistency (1 - viscous, 2 - viscous or frothy bloat and 3 - lightly viscous).
[10]Sedimentation and flotation time (1 - active (0 to 4 min), 2- normal (4 to 8 min) and 3 - reduced (greater than 8 min).
[11]Potential redox (1 - active (0 to 3 min); 2 - normal (3 to 5 min) and 3 - reduced (greater than 5 min)).
[12, 13, 14, 15]Microbiological protozoa (1 - absent, 2 - little, 3 - normal, 4 - abundant).
Source: Authors.

Ruminal fluid color and odor were not influenced by NA in the diet (P > 0.05), with all animals presenting olive green fluid and an aromatic odor indicative of ruminal health. Regarding consistency, treatment NA60 presented greater viscosity (P < 0.05) of content compared to the other groups, which presented a more aqueous content (P < 0.05).

The ruminal fluid of NA15 and NA30 animals had a longer sedimentation time (P < 0.05) (4 to 8 min) than that of the other groups (0 to 4 min).

According to the redox potential tests, the ruminal fluid of animals in treatment NA30 presented a more active metabolism than those in CON and NA15, whose activities were closer to those of normal metabolization ($P < 0.05$). Values for all other treatments were similar, at around 1.2. However, although all the parameters evaluated in this study indicated healthy rumen function, and thus the addition of NA to the diet did not affect the ruminal environment, it did induce pathological changes such as the defaunation of microflora. This finding correlates with those observed by Sallam et al. (2011) for the addition of citrus essential oil (0.5 and 0.75 mg/d) and its secondary metabolite limonene (0.45 and 0.60 mg/d). The *in vitro* study carried out by Cieslak et al. (2009) also confirmed the potential of limonene to inhibit the power of protozoa (at 40 or 400 mg/L), while Wanapat et al., 2008) observed similar results for the addition of lemon grass essential oil (at 100, 200 or 300 g/d).

Microbiological protozoa populations were not influenced by the inclusion of the selected OB in the steer diet (*P > 0.05*), with an average total count of 242.1x10³/mL and mean percentages of viable protozoa of 66, 72, 76, 80, and 84% in treatments CON, NA15, NA30, NA45, and NA60, respectively. However, an increasing tendency in the percentage of viable protozoa was recorded at higher NA levels ($P = 0.10$). The average density of protozoa was 1.5 points, a value classified as abundant to moderate. Based on these data, no defaunation was observed, a phenomenon closely related to an increase in ruminal transit rate and an increase in the metabolism of bacterial protein.

Populations were dominated by large protozoa (1.6 points – abundant to moderate), followed by medium (2.92 points – moderate) and small protozoa at lower frequencies (3.0 points – low). No significant differences were recorded between the counts of any groups, indicating that the presence of the EOB did not impair ruminal fauna, and was not toxic to any specific group of protozoa. Thus, the inclusion of the selected EOB in the steer diet did not alter any of the microbiological parameters evaluated. These results are similar to those of Newbold, 2004, who also found no influence of oil blend use on protozoa numbers.

4. Final Considerations

The results suggest that the use of a mixture of oil blend for dietary supplementation in grazing cattle did not modify the animals' body weight gain, but did alter food intake and digestibility. An increase in the concentration of rumen ammoniacal nitrogen was also recorded, as well as in propionic and isovaleric volatile fatty acids. No marked effects were observed in the microbiological population of the rumen. These results suggest that doses

above 1500 mg/animal/d are high for livestock grazing on temperate pasture and that studies conducted using doses below this value are required.

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