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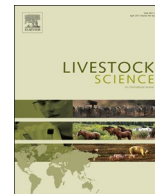
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# Effects of diet supplementation with clove and rosemary essential oils and protected oils (eugenol, thymol and vanillin) on animal performance, carcass characteristics, digestibility, and ingestive behavior activities for Nellore heifers finished in feedlot

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## ABSTRACT

This study was carried out to evaluate the influence of essential oils and their blends on animal performance, feed intake, *in situ* digestibility, ingestive behavior activities, and carcass characteristics for heifers finished in feedlot on a high-grain diet (~65% corn, 25% corn silage, 10% soybean meal). Forty Nellore heifers (initial body weight  $297.6 \pm 31.2$  kg) were used in the experiment and distributed randomly among individual pens. Dietary treatments based on essential oil additives included: CON – Without essential oil; ROS – Rosemary essential oil; BLE – Protected blend of eugenol, thymol, and vanillin; BCL – Protected blend + clove essential oil; and BRC – Protected blend + rosemary essential oil + clove essential oil. There were no diet effects on initial and final body weights. However, average daily gains, dry matter intakes (kg/d), and dry matter intakes (%BW) were greater ( $P < 0.05$ ) in heifers fed with BLE, BCL, and BRC diets than in heifers fed with ROS diets. Feed efficiency (gain to feed) was greater ( $P < 0.0001$ ) in heifers fed the BCL and BRC diets when compared to heifers fed the ROS diet. There were no diet effects on carcass characteristics. *In situ* digestibility of dry matter and neutral detergent fiber were greater ( $P < 0.0001$ ) in heifers fed the three blended diets when compared to heifers fed the ROS diet. The addition of essential oils to the diets of heifers did not alter the muscle, fat, or bone percentages in the carcass. For ingestive behavior activities, data on rumination and idleness tended to be altered by diet with increased rumination in heifers fed BRC diet. The addition of 4 g/animal/d of a blend of essential oils to the diets of Nellore heifers improved average daily gain, dry matter intake, feed efficiency, and ingestive behavior activities.

## 1. Introduction

In Brazil, the traditional beef production systems are extensive and pasture based, with Zebu breeds (*Bos taurus indicus*), such as Nellore and European crossbreeds (*Bos taurus* × *B. taurus indicus*) frequently used (Rotta et al., 2009). In recent years, increases in domestic and export beef demand have resulted in use of more intensive production systems which feed a high percentage of concentrates to meet market demand (Prado et al., 2008).

The addition of antibiotics to livestock production systems has been common, especially when animals are reared intensively, in order to prevent diseases and metabolic disorders and improve feed efficiency. However, the use of antibiotics has been banned in some regions due to the emergence of bacteria resistant to antibiotics and the possible risks to human health from possible residues in the final products (Russell and Houlihan, 2003). Thus, the livestock industry is seeking alternative solutions, including the use of essential oils as a potential alternative/substitute for antibiotics to improve cattle performance

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(Cruz et al., 2014).

The essential oils are liquid, aromatic extracts due to the volatile nature of the components extracted from plant materials, such as flowers, buds, seeds, leaves, twigs, barks, wood, fruit, and roots. They may be obtained by fermentation, extraction, or most commonly, by steam distillation (Burt, 2004). Chemically, essential oils are variable mixtures of terpenoids that primarily include monoterpenes (C<sup>10</sup>) and sesquiterpenes (C<sup>15</sup>), although diterpenes (C<sup>20</sup>) may also be present. They also include a variety of low-molecular-weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, or lactones. Other compounds found in essential oils include coumarins and homologues of phenylpropanoids. These products act as antimicrobial and antioxidant agents which have been found to benefit the immune and digestive systems of animals and improve animal performance (Jayasena and Jo, 2013).

Interest in the use of essential oils as a potential substitute for antibiotics in cattle diets has been developed from the results of *in vitro* studies (Meyer et al., 2009) showing that essential oils have antimicrobial activity against the microflora present in the gastrointestinal tract. There has been limited research to date since the actions of essential oils are dependent on their chemical composition, the doses used, and the synergistic effects among chemical compounds in the oils.

This study was carried out to evaluate the effects of essential oils and their blends as an antimicrobial alternative in the finishing phase for feedlot cattle. Animal performance (gain, feed intake, feed efficiency), carcass characteristics, *in situ* digestibility, and ingestive behavior activities were examined for Nelore heifers supplemented with (without) essential oils over a 73-day feeding period.

## 2. Materials and methods

This experiment was approved by the Committee for Ethics in the use of Animals (CEUA) of the Universidade Estadual de Maringá, following protocol 3624,120,116.

### 2.1. Animals and treatments

The experiment was carried out at Sector Rosa & Pedro at the experimental farm of Universidade Estadual de Maringá, Paraná, Brazil. Forty Nelore purebred heifers with a mean initial body weight (BW) of 297.6 ± 31.2 kg were used in this study. Heifers were distributed randomly in individual pens, with dimensions of 10 m<sup>2</sup> for each animal, partially covered and equipped with masonry automatic drinkers and

feeders. The period of adaptation to the feedlot and concentrate diet was 7 days; afterwards, the cattle were fed for 73-days until animals reached a mean BW of 356.6 ± 32.6 kg. During the experimental period, Nelore heifers were weighed monthly in order to record weight gain.

Nelore heifers were randomly assigned to one of five dietary treatments with eight heifers per treatment group. Dietary treatments included: CON – Without essential oil; ROS – Rosemary essential oil (4 g/animal/d); BLE – Protected blend of eugenol, thymol, and vanillin (4 g/animal/d); BCL – Protected blend – eugenol, thymol, and vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d); and BRC – Protected blend – eugenol, thymol, and vanillin (1.33 g/animal/d) + rosemary essential oil (1.33 g/animal/d) + clove essential oil (1.33 g/animal/d).

The rosemary and clove essential oils were liquids, obtained from FERQUIMA<sup>®</sup> (Vargem Grande Paulista, São Paulo, Brazil). The essential oil blend (eugenol, thymol, and vanillin) was in a powder form and was obtained from Safeeds<sup>®</sup> (Cascavel, Paraná, Brazil). These plant extracts were chosen based on antioxidant potential (Biondo et al., 2016), while the dosage was determined based on past studies (Benchaar et al., 2006a, 2006b; Busquet et al., 2006).

The feedlot diets were based on ad libitum feeding of corn grain (647 g/kg of DM) and corn silage (250 g/kg of DM). Preparation of diets was made with a premix of essential oils with soybean meal; this premix was then added to the feed mixer with other dietary ingredients. Soybean meal (100 g/kg of DM) was mixed with yeast (0.40 g/kg of DM), phosphorus (0.70 g/kg of DM) mineral salt (1.90 g/kg of DM), essential oils (4 g/animal/d) and top-dressed daily into the morning feeding for respective essential oil treatment pens (ROS, BLE, BCL, and BRC) during the experimental period. Soybean meal mixture was also top-dressed into the morning feeding for CON pens during the experimental period, without the addition of essential oils. The diets were analyzed by the oxygen radical absorbance capacity (ORAC) method as reported by Zulueta et al. (2009), since the antioxidant activities of dietary essential oils remain active for up to 30 days of exposure. All diets were isonitrogenous, isoenergetic, and formulated to meet the requirements for a gain of 1.0 kg/d (NRC, 2000) with adequate concentrations of nutrients for the growth and finishing of animals. Diets with essential oils were prepared every 15 days; however, diet formulations were adjusted based on the intake of dry matter (DM)/d per animal determined on a monthly basis when the cattle were weighed.

**Table 1**

Chemical composition of dietary ingredients used in basal diet with no added essential oils (g/kg of DM).

Ingredients	DM <sup>a</sup>	OM <sup>b</sup>	Ash	CP <sup>c</sup>	EE <sup>d</sup>	NDF <sup>e</sup>	ADF <sup>f</sup>	TC <sup>g</sup>	NFC <sup>h</sup>	ME <sup>i</sup>
Corn silage	306	969	30.9	71.1	27.1	424	224	870	446	2.34
Corn grain	853	984	16.4	96.1	47.1	175	45.8	840	665	3.00
Soybean meal	850	933	67.0	489	19.0	159	87.8	425	266	3.13
Yeast	920	954	46.1	331	21.0	26.0	9.22	572	546	–
Phosphorus	995	38.0	962	–	–	–	–	–	–	–
Mineral salt <sup>j</sup>	986	55.0	945	–	–	–	–	–	–	–
Diet	716	973	27.1	129	39.1	235	94.4	804	568	2.84

<sup>a</sup> Dry matter.

<sup>b</sup> Organic matter.

<sup>c</sup> Crude protein.

<sup>d</sup> Ether extract.

<sup>e</sup> Neutral detergent fiber.

<sup>f</sup> Acid detergent fiber.

<sup>g</sup> Total carbohydrates.

<sup>h</sup> Non-fiber carbohydrates.

<sup>i</sup> Metabolizable energy (Mcal/kg).

<sup>j</sup> Mineral salt composition (kg): calcium, 50 g; magnesium, 57 g; sodium, 81 g; sulfur, 3.75 g; cobalt, 20 mg; copper, 500 mg; iodine, 25 mg; manganese, 1.500 mg; selenium, 10 mg; zinc, 2.000 mg; vitamin A, 400.000 UI; vitamin D3, 50.000 UI; vitamin E, 750 UI; ether extract, 168 g; urea, 200 g.

## 2.2. Chemical analyses

The chemical composition of ingredients and experimental diets are presented as g/kg of DM (Table 1). DM was determined after oven drying at 65 °C for 24 h and milling through a 1-mm screen following method ID 934.01 (AOAC, 2005). Ash content was measured by combustion at 550 °C for 16 h according to method ID 942.05 (AOAC, 2005). Nitrogen concentration was determined by the Kjeldahl method (ID 988.05) (AOAC, 2005). Following the determination of nitrogen concentration, crude protein was calculated by multiplying the nitrogen content by a factor of 6.25. Ether extract content was determined by method ID 920.39 (AOAC, 2005). The neutral detergent fiber (NDF) content was measured according to the recommendations of Mertens (2002) using  $\alpha$ -amylase and was expressed inclusive of residual ash. The acid detergent fiber (ADF) content was measured by using method ID 973.18 (AOAC, 2005) and was expressed inclusive of residual ash. Total carbohydrates were estimated by the procedure of Sniffen et al. (1992) as follows. Non-fibrous carbohydrates were determined as the difference between total carbohydrates and NDF. Metabolizable energy content of feedstuffs was estimated according to NRC (2000) recommendations.

## 2.3. Feed intake, growth performance, and carcass characteristics

Diets were offered at 08:00 and 16:00 h every day. Feed intake was estimated as the difference between the feed supplied and refusals in the trough. Feed efficiency was calculated as the ratio between average daily gain and DM intake. To determine growth performance, animals were weighed at the beginning of the experiment and then every month (after fasting for 16 h), throughout the experiment. The average daily gain was calculated as the total BW gain divided by the length of the experimental period (73-days).

The Nellore heifers were slaughtered in a commercial slaughterhouse, 130 km from the Iguatemi Experimental Farm after 73-days of feeding the experimental diets when cattle reached a mean final body weight of  $356.6 \pm 32.6$  kg. Animal transport was carried out in the late afternoon to minimize stress. Upon arrival at the slaughterhouse, animals were kept in resting pens and were subsequently stunned using a penetrating captive bolt pistol as per Brazilian federal inspection regulations according to the Brazilian RIISPOA – Regulation of Industrial and Sanitary Inspection of Animal Products.

After slaughter, the carcasses were identified, weighed, and chilled for 24 h at 4 °C. The cold carcass weight was determined after chilling. The carcass dressing percentage (hot and cold) was calculated by applying the following equation:

$$CDP = CW \times 100 / FBW \quad (1)$$

where: CDP, CW, and FBW are Carcass dressing percentage; Carcass weight; and Final body weight, respectively.

Carcass shrink was determined by measuring the difference between the weight obtained before and after refrigeration for 24 h ( $\pm 4$  °C).

## 2.4. Carcass tissue composition

Carcass composition was determined/estimated by dissection of the 6th rib according to the methodology of Robelin and Geay (1975). Muscle, fat (subcutaneous and inter-muscular), bone, and other tissues (tendons and fascia) were separated.

## 2.5. In situ digestibility

The determination of total digestibility using the indicator, indigestible neutral detergent fiber (iNDF) was carried out according to the methodology described by Zeoula et al. (2002). Samples of feed, feces, and refusals were incubated in rumen cannulated heifers using F57 filter bags for 288 h (Ankom Technology, NY, USA) with

dimensions of 5.0 × 5.0 cm and a porosity of 50 mm. A 1.0-g sample was incubated for concentrates and 0.5 g for silage, feces, and refusals. Following removal of the bags from the rumen, they were washed by hand under running water until the resulting wash water became clear. The bags were subsequently placed to dry in a forced air ventilation oven at 60 °C for 48 h and then boiled in a neutral detergent solution (TE-149, Tecnal, SP, Brazil) to determine iNDF content.

Fecal flow was determined using the following equation:

$$FF = IC / CIF \quad (2)$$

where: FF, IC, and CIF are Fecal flow; Indicator consumed; and Concentration indicator in feces, respectively.

The digestibility coefficient was calculated by the following equation:

$$DC = (NI - NE) / NI \quad (3)$$

where: DC, NI, and NE are Digestibility coefficient; Nutrient intake; and Nutrient excreted, respectively.

## 2.6. Ingestive behavior activities

Data on feeding behavior were obtained between the 6th and 7th weeks of feeding the experimental diets. The record of time spent on different activities was obtained by visual observation of the animals every 5 min, carried out by a trained team over 24 uninterrupted hours (Silva et al., 2006). Data were collected to estimate the duration of periods spent feeding, drinking, ruminating, and idle. The total time spent on each activity was determined by the sum of repetitions.

The parameters of feed efficiency and rumination efficiency on DM and NDF were determined according to the adapting the methodology proposed by Bürger et al. (2000), using to the equations described below:

$$\begin{aligned} FE_{DM} &= DMI / FD \\ FE_{NDF} &= NDFI / FD \\ RE_{DM} &= DMI / RUD \\ RE_{NDF} &= NDFI / RUD \end{aligned} \quad (4)$$

where:  $FE_{DM}$  = Feeding efficiency of dry matter (kg DM/h); DMI = Dry matter intake (kg DM/d); FD = Feeding duration (h/d);  $FE_{NDF}$  = Feeding efficiency of neutral detergent fiber (kg NDF/h); NDFI = Neutral detergent fiber intake (kg NDF/d);  $RE_{DM}$  = Rumination efficiency of dry matter (kg DM/h); RUD = Rumination duration (h/d);  $RE_{NDF}$  = Rumination efficiency of Neutral detergent fiber (kg NDF/h).

## 3. Statistical analyses

Data were analyzed by using the ANOVA procedure of SAS (SAS, 2004) to perform a randomized complete experiment with five diets and eight replications. The model included the fixed effects of essential oil diets according the following equation:

$$Y_{ij} = \mu + T_i + e_{ij} \quad (5)$$

where:  $Y_{ij}$ ,  $\mu$ ,  $T_i$ ,  $e_{ij}$  are Dependent variables; Mean value common to all observations; Fixed effect of essential oils diets; and the error term, respectively.

For each studied variable, the mean and standard error of the mean (SEM) were calculated and differences between means were evaluated using Duncan's Multiple Range Test ( $P \leq 0.05$ ).

## 4. Results

Final body weights (FBW) were not affected ( $P > 0.05$ ) by essential oil addition to the diets (Table 2). Average daily gains (ADG) were significantly greater ( $P < 0.001$ ) for heifers fed BCL and BRC diets than for heifers fed CON and ROS diets. The ROS dietary treatment had the lowest ( $P < 0.0001$ ) dry matter intakes (DMI) and feed efficiency when

**Table 2**

Effect of diets with (without) inclusion of essential oils on animal performance, feed efficiency and carcass characteristics of Nellore heifers finished in feedlot.

Parameters	CON <sup>a</sup>	ROS <sup>b</sup>	BLE <sup>c</sup>	BCL <sup>d</sup>	BRC <sup>e</sup>	SEM <sup>f</sup>	p-value
Initial body weight (kg)	292	311	289	290	306	4.93	0.50
Final body weight (kg)	343	346	356	361	377	5.16	0.28
Average daily gain (kg/d)	0.70 <sup>b</sup>	0.47 <sup>c</sup>	0.91 <sup>a,b</sup>	0.97 <sup>a</sup>	0.97 <sup>a</sup>	0.04	0.02
Dry matter intake (kg)	5.49 <sup>b,c</sup>	5.07 <sup>c</sup>	6.25 <sup>a</sup>	5.90 <sup>a,b</sup>	6.21 <sup>a</sup>	0.13	0.01
Dry matter intake (% Initial body weight)	1.74 <sup>b</sup>	1.55 <sup>c</sup>	1.95 <sup>a</sup>	1.82 <sup>a,b</sup>	1.82 <sup>a,b</sup>	0.04	0.01
Feed efficiency <sup>g</sup>	0.13 <sup>b</sup>	0.09 <sup>c</sup>	0.15 <sup>a,b</sup>	0.16 <sup>a</sup>	0.16 <sup>a</sup>	0.02	0.01
Hot carcass weight (kg)	186	190	191	193	203	2.84	0.37
Cold carcass weight (kg)	183	186	186	188	198	2.86	0.54
Hot dressing carcass (%)	54.0	54.7	53.7	53.4	54.0	0.19	0.32
Cold dressing carcass (%)	53.1	53.6	52.4	52.2	52.5	0.24	0.41
Carcass shrink (%)	1.62	1.58	1.50	1.58	1.86	0.05	0.19

<sup>abc</sup>Values with different letters in the same row are different by Duncan test ( $P \leq 0.02$ ).

<sup>a</sup> CON – Without essential oil.

<sup>b</sup> ROS – Rosemary essential oil (4 g/animal/d).

<sup>c</sup> BLE – Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

<sup>d</sup> BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

<sup>e</sup> BRC – Protected blend protected – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d), clove essential oil (1.33 g/animal/d).

<sup>f</sup> SEM: Standard error of means.

<sup>g</sup> kg average daily gain/kg dry matter feed intake.

**Table 3**

Effect of diets with (without) essential oils on carcass composition of Nellore heifers finished in feedlot.

	Diets						
	CON <sup>a</sup>	ROS <sup>b</sup>	BLE <sup>c</sup>	BCL <sup>d</sup>	BRC <sup>e</sup>	SEM <sup>f</sup>	p-value
Muscle	55.7	55.1	55.2	58.2	55.4	1.20	0.81
Fat	24.8	24.8	25.8	21.8	24.6	1.26	0.52
Bone	17.6	17.7	16.5	17.5	17.6	0.66	0.97
Other	1.85	2.39	2.44	2.42	2.42	0.24	0.39

<sup>a</sup> CON – Without essential oil.

<sup>b</sup> ROS – Rosemary essential oil (4 g/animal/d).

<sup>c</sup> BLE – Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

<sup>d</sup> BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

<sup>e</sup> BRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d), clove essential oil (1.33 g/animal/d).

<sup>f</sup> SEM: Standard error of means.

compared to the other essential oil treatments. While the lowest feed efficiency was found in heifers fed the ROS diet, there were no differences in feed efficiency between heifers fed the CON and BLE diets.

Hot and cold carcass weights and dressing percentages and carcass shrink were not affected ( $P > 0.05$ ) by the addition of essential oils to the heifer diets (Table 2). There were no differences ( $P > 0.05$ ) in body

**Table 4**Effect of diets with (without) essential oils on *in situ* digestibility (g/kg of DM).

	CON <sup>a</sup>	ROS <sup>b</sup>	BLE <sup>c</sup>	BCL <sup>d</sup>	BRC <sup>e</sup>	SEM <sup>f</sup>	p-value
Dry matter	0.65 <sup>a</sup>	0.62 <sup>b</sup>	0.64 <sup>a</sup>	0.66 <sup>a</sup>	0.65 <sup>a</sup>	0.04	0.01
Crude protein	0.71	0.68	0.70	0.71	0.71	0.06	0.97
Neutral detergent fiber	0.47 <sup>b</sup>	0.45 <sup>c</sup>	0.47 <sup>b</sup>	0.48 <sup>a</sup>	0.47 <sup>b</sup>	0.02	0.01

<sup>abc</sup>Values with different letters in the same row statistically different by Duncan test ( $P = 0.01$ ).

<sup>a</sup> CON – Without essential oil.

<sup>b</sup> ROS – Rosemary essential oil (4 g/animal/d).

<sup>c</sup> BLE – Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

<sup>d</sup> BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

<sup>e</sup> BRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d), clove essential oil (1.33 g/animal/d).

<sup>f</sup> SEM: Standard error of means.

**Table 5**

Effect of diets with (without) essential oils on ingestive behaviour activities of Nellore heifers finished in feedlot.

Activities	CON <sup>a</sup>	ROS <sup>b</sup>	BLE <sup>c</sup>	BCL <sup>d</sup>	BRC <sup>e</sup>	SEM <sup>f</sup>	p-value
Drinking (No. visits)	4.00	3.06	2.75	4.43	2.25	0.35	0.29
Feeding (No. visits)	17.3	22.2	19.1	24.31	22.4	1.24	0.41
Rumination time (hours)	198 <sup>b</sup>	215 <sup>b</sup>	231 <sup>a,b</sup>	216 <sup>b</sup>	274 <sup>a</sup>	8.89	0.07
Idleness time (hours)	1135 <sup>b</sup>	1098 <sup>a,b</sup>	1099 <sup>a,b</sup>	1080 <sup>a,b</sup>	1042 <sup>a</sup>	11.0	0.09
FE <sub>DM</sub> (kg DM/h) <sup>g</sup>	6.22	4.94	5.63	4.87	5.19	0.36	0.77
FE <sub>NDF</sub> (kg DM/h) <sup>h</sup>	1.57	1.25	1.42	1.23	1.31	0.09	0.77
RE <sub>DM</sub> (kg DM/h) <sup>i</sup>	2.40	2.05	2.51	2.50	1.94	0.10	0.21
RE <sub>NDF</sub> (kg DM/h) <sup>j</sup>	0.61	0.52	0.63	0.63	0.49	0.03	0.21

<sup>ab</sup>Values with different letters in the same row statistically different by Duncan test.

<sup>a</sup> CON – Without essential oil.

<sup>b</sup> ROS – Rosemary essential oil (4 g/animal/d).

<sup>c</sup> BLE – Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

<sup>d</sup> BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

<sup>e</sup> BRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d), clove essential oil (1.33 g/animal/d).

<sup>f</sup> SEM: Standard error of means.

<sup>g</sup> FE<sub>DM</sub>: Dry matter feeding efficiency.

<sup>h</sup> FE<sub>NDF</sub>: Neutral detergent fiber feeding efficiency.

<sup>i</sup> RE<sub>DM</sub>: Dry matter rumination efficiency.

<sup>j</sup> RE<sub>NDF</sub>: Neutral detergent fiber rumination efficiency.

composition (% muscle, fat, bone) across dietary treatments based on dissection of the 6th rib from the carcass (Table 3).

The addition of essential oils and their blends to the diet affected ( $P < 0.05$ ) *in situ* digestibility of DM and neutral detergent fiber (NDF) (Table 4). *In situ* digestibility values for DM and NDF were lower in heifers fed the ROS diet relative to the other diets. For ingestive behavior activities, data on rumination and idleness tended to be altered by diet ( $P < 0.10$ ; Table 5). Feeding and drinking were not affected ( $P > 0.05$ ; Table 5) by the addition of essential oils to the diet.

## 5. Discussion

Heifers in the present study were slaughtered at a FBW in



accordance with the Nellore standard and Brazilian slaughterhouses requirements, which advocate a final body weight for heifers from 320 to 380 kg (Ferraz and Felício, 2010). Inclusion of only rosemary essential oil as an added essential oil to the diet resulted in the lowest gains, whereas supplementation with essential oil treatments (BCL and BRC) increased ADG versus heifers fed the CON diet. The low weight gains for heifers fed CON and ROS diets were due to lower feed intakes in general versus heifers fed the other diets with a blend of essential oils.

While the lowest DMI values were fed with heifers fed ROS diet, DMI values were much greater when rosemary essential oil was mixed with other essential oils in the BRC diet. Plants develop defense mechanisms, and may, use constituent chemical compounds to defend against herbivorism (Gershenzon and Croteau, 1991). Volatile chemical compounds found in essential oils include, camphor, limonene,  $\alpha$ -pinene,  $\beta$ -carophyllene, p-cymene,  $\alpha$ -humulene, and others (Burt, 2004). Working with isolated camphor and carophyllene compounds, Estell et al. (1998) observed that DMI in sheep decreased by 14% and 16% respectively versus feeding the control diet. The essential oil from rosemary is rich in volatile compounds including 1.8 cineole,  $\alpha$ -pinene,  $\beta$ -carophyllene, camphene, camphor, and borneol (Smeti et al., 2013), which could affect DMI when applied with other essential oils that have similar compounds.

The poor feed efficiency for heifers fed the ROS diet may have been due to poor palatability of the diet which limited DMI and ADG. The best values for feed efficiency in heifers were found for diets with blends of essential oils, due to a possible synergism from mixing essential oils. This effect was probably due to a ruminal environment appropriate (pH 5.5), promoted by highly concentrate diets, providing the best activity/ action of the molecules present in each essential oil, which reflects positively on animal production. This is supported by Cardozo et al. (2005) who found that adding a blend of cinnamaldehyde and eugenol to a diet that promotes an acidic rumen environment enhances animal performance (better weight gains and feed efficiency), whereas animal performance is not affected when the additives are supplemented in diets where rumen pH is neutral.

The hot and cold carcass weights values in the present study were in accordance with Nellore and industry standards which advocate weights for hot carcass of heifers from 180 to 200 kg (Ferraz and Felício, 2010). The average hot and cold carcass dressing percentages were superior to, Nellore and industry standards where a 52% carcass dressing percentages is considered normal for Nellore heifers slaughtered at 24 months of age, and for other studies evaluating heifers slaughtered at similar body weights (Marques et al., 2010; Farias et al., 2012). In general, carcass shrink losses in industry range from 1.5% to 2.0% after 24 h of chilling which is in agreement with the present study. Thus, the losses observed in this experiment are consistent with losses considered normal (Andreotti et al., 2015). Feedlot studies carried out with crossbred heifers in feedlot have reported muscle, fat, and bone percentages ranging from 56% to 62%, 20% to 25%, and 16% to 19%, respectively (Andreotti et al., 2015). Thus, the muscle, fat, and bone percentages obtained in this study can be considered normal for these animal categories.

According to Oh et al. (1968), the low palatability of some natural extracts to ruminants such as rosemary essential oil in the present study may not only be due to sensory effects but also to effects on microbial flora, directly affecting total DM digestibility. Nagy and Tengerdy (1968) evaluated the sensitivity of ruminal microorganisms to the essential oil, *Artemisia tridentate* on captive and wild deer. The authors found that by increasing the amounts of the essential oils of *Artemisia tridentate*, from 0 (no essential oils) to 20 < mu > L/ 10 ml of medium, rumen bacteria counts after 24 h of incubation decreased from  $4.6 \times 10^9$  to  $5.7 \times 10^2$ . This essential oil contains 1.8-cineole as its primary compound which is also found in essential oil of rosemary. There is evidence that high intake of *Artemisia tridentate* caused digestive problems in ruminants. In addition, high doses of this oil reduced

total viable bacteria counts when added to *in vitro* cultures of rumen bacteria (Nagy and Tengerdy, 1968). In this study, use of rosemary essential oil as the only added source of essential oils reduced total digestibility of DM and NDF.

The reason for similar values for ingestive behavior activities is most likely due to all animals receiving a basal diet in which there were no major differences in dietary ingredients, as fiber content and particle size are the main factors involved for influencing ingestive behavior (Mendes Neto et al., 2007). However, essential oils are able to reduce protein degradation, causing a reduction in adherence and colonization of bacteria with proteolytic activity (Benchaar et al., 2008); consequently, rumination rates increase in order to reduce particulate ingredients found in the rumen. This affirmation about reduction in protein degradation is contested by the results found in the present study, since no difference was observed with the technique used (*in situ*).

The inclusion of essential oils in the diet (BRC treatment) resulted in an increased rumination rate, while there was a decrease in the idleness rate (Table 5), thus demonstrating that essential oils can positively influence animal production (Marques et al., 2008). These values for feeding and drinking are very important because such extracts have a rather sharp odor and taste and can be used as stimulators of consumption, a factor that was not observed in the present study. According to Yang et al. (2010), very high doses of essential oils administered in the diet can influence feed consumption differently as compared to feeding low doses.

## 6. Conclusions

The present results suggest that the use of a blend of 4 g/animal/d of natural additives in the diets of Nellore heifers improves animal performance; blends like BCL and BRC improve the gains and feed efficiency, while blend BLE improve the feed intake. Use of rosemary essential oil on its own tended to decrease animal performance; this contrasts to improvements in animal performance when rosemary essential oil is added to the diet as a blend of essential oils. The blend of clove essential oil (2 g/animal/d) and protected oils [eugenol, thymol, and vanillin (2 g/animal/d)] proved to be promising for improving animal performance.

## Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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