A blend of essential oils improved feed efficiency and affected ruminal and systemic variables of dairy cows

Rayana Brito da Silva,[*,†] Marcos Neves Pereira,[*,†,1] Rafael Canonenco de Araujo,[‡] Wesley de Rezende Silva,[*] and Renata Apocalypse Nogueira Pereira,[†,§]

[*]Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, MG 37200-000, Brazil; [†]Better Nature Research Center, Ijaci, MG 37218-000, Brazil; [‡]GRASP Indústria e Comércio Ltda, Curitiba, PR 81260-000, Brazil; and [§]Empresa de Pesquisa Agropecuária de Minas Gerais, Unidade Regional Sul de Minas, Lavras, MG 37200-000, Brazil

ABSTRACT: This experiment evaluated the effect of a blend of essential oils (**BEO**) on intake, lactation performance, diet digestibility, ruminal fermentation profile, eating behavior, body thermoregulation, blood acid–base balance, and milk fatty acid profile of lactating cows. Twenty-eight Holstein cows were individually fed a standard diet for 14 d and treatments control or BEO (a microencapsulated blend of pepper extract containing capsaicin and pure forms of carvacrol, cinnamaldehyde, and eugenol; 150 mg/kg of diet dry matter) for 56 d. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. Dry matter intake (**DMI**) was reduced (20.1 vs. 19.5 kg/d) and milk yield was increased (30.1 vs. 30.8 kg/d) by BEO, inducing improved milk to DMI ratio (1.53 vs. 1.62). Milk fat concentration tended to be increased by BEO, but total solids yield did not differ. There was a trend for increased total tract non-neutral detergent fiber organic matter digestibility with BEO. The molar proportion of acetate in ruminal fluid was reduced (57.8 vs. 51.4%) and that of propionate was increased (26.1 vs. 31.3%) by BEO. Ruminal microbial yield and total protozoa count in ruminal fluid did not differ. Cows fed BEO ingested a greater proportion of the daily intake in the morning (30.6 vs. 36.6%) and tended to ingest a lower proportion at night, tended to have longer meals, and had fewer meals per day (13.7 vs. 11.9 kg/d) and larger meal size (1.5 vs. 1.7 kg of dry matter per meal). Blood urea-N and glucose concentrations did not differ. The BEO increased jugular blood oxygenation. The sweating rate on a hot and dry day was increased (160 vs. 221 g/m^2 per h) by BEO. The mean rectal and skin temperatures and respiration rate did not differ, but the proportion of rectal temperature measurements ≥39.2 °C was reduced by BEO at 1400 h (28.5 vs. 17.8%) and 2000 h (34.8 vs. 23.2%). The BEO increased the secretion (g/d) of 18:2 *trans*-10, *cis*-12 and the concentration of 18:0 *iso* fatty acids in milk fat. When one sample of milk from BEO cows was offered with two samples of milk from control, 59% of regular consumers of milk ($n = 63$) identified the odd sample correctly. The gain in feed efficiency induced by BEO was associated with reduced acetate-to-propionate ratio in ruminal fluid, altered eating behavior, lower frequency of high rectal temperature, and increased blood oxygenation. Essential oils had positive effects on ruminal fermentation and systemic variables of dairy cows.

Key Words: digestibility, eating behavior, essential oils, feed efficiency, ruminal fermentation, thermoregulation

© The Author(s) 2019. Published by Oxford University Press on behalf of the American Society of Animal Science.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Transl. Anim. Sci. 2020.4:182–193
doi: 10.1093/tas/txz183

[1]Corresponding author: mpereira@ufla.br
Received September 17, 2019.
Accepted December 16, 2019.

INTRODUCTION

Plant-derived bioactive compounds, such as essential oils (**EO**), represent a wide range of mainly volatile compounds with a potential to manipulate ruminal microbial population and fermentation profile by diverse modes of action (Calsamiglia et al., 2007). It has been demonstrated that EO can reduce the ruminal acetate-to-propionate ratio, AA deamination, and methanogenesis (Calsamiglia et al., 2007; Benchaar et al., 2008a; Klevenhusen et al., 2012). In addition to their effects on rumen microbes, EO may affect the physiology of the host ruminant (Oh et al., 2013). Synergistic and additive effects may occur among various EO (García-García et al., 2011), justifying the use of blends instead of single-component products as feed additives (Hashemzadeh-Cigari et al., 2014). Some studies performed with dairy cows have reported improvements in milk yield (Hashemzadeh-Cigari et al., 2014), feed efficiency (Tassoul and Shaver, 2009; Hristov et al., 2013), and milk composition (Santos et al., 2010) in response to EO, although the detection of positive lactation response to EO supplementation is not consistent (Benchaar et al., 2008b; Spanghero et al., 2009; Tager and Krause, 2011; Tekippe et al., 2013). The evaluation of the effect of EO on dairy cow intake and lactation performance is scarce and warranted (Khiaosa-ard and Zebeli, 2013). This experiment evaluated the effect of a microencapsulated blend of EO (**BEO**; carvacrol, cinnamaldehyde, eugenol, and capsaicin) on feed intake, lactation performance, feed efficiency, diet digestibility, ruminal fermentation profile, eating and rumination behavior, body thermoregulation, blood acid−base balance, and milk fatty acid profile of lactating cows. In addition, a sensory evaluation of the milk produced by cows fed BEO was performed. We hypothesized that BEO would have positive effects on lactation performance and feed efficiency mediated by changes in ruminal fermentation profile and systemic physiology.

MATERIALS AND METHODS

The experimental protocol was approved by the Ethics Committee on Animal Use of the University of Lavras.

Housing, Cows, Diet, and Experimental Design

The experiment was conducted in an open-walled, sand-bedded tie-stall barn with fans and high-pressure sprinklers during the transition from the dry to the rainy season of Brazil's central region (October to December). The research center is located at 846 m above sea level, lat 21°09′52.41″S, and long 44°55′52.40″W. Environmental temperature and humidity at the center of the barn were measured at 30-min intervals. Temperature was 22.7 ± 4.2 °C and humidity was 82.0% ± 14.4% (mean ± SD; 2,688 recordings). The temperature–humidity index (**THI**) was calculated according to Yousef (1985). The THI was 70.8 ± 4.68; THI > 68: 68.6% of time.

Twenty-eight Holstein cows (181 ± 102 d in milk, 10 primiparous) were individually fed the same total mixed ration (**TMR**) for a 14-d standardization period. On days 9 to 14 of this period, dry matter (**DM**) intake (**DMI**), milk yield, milk solids, body weight (**BW**), and body condition score (**BCS**) were measured to be used as covariates in the statistical model. Cows were blocked by parity (1 vs. ≥2) and milk yield, and assigned to a treatment for 8 wk. Treatments were control (no feed additive) or BEO (150 mg of product/kg of TMR DM). The product (Activo Premium, GRASP Ind. & Com. LTDA, Curitiba, Brazil) is a blend of pepper extract containing capsaicin and pure forms of carvacrol, cinnamaldehyde, and eugenol. Active components represent 15% of the product and are microencapsulated in a fat matrix. The feed additive was included in the vitamin and mineral premix and was added to the TMR mixed in a stationary feed mixer (Unimix 1200; Casale, São Carlos, Brazil) offered at 0700 and 1300 h in amounts to allow for 7% to 10% of offered as daily refusal per cow. Feed was pushed up at least 10×/d. Cows were milked 2×/d starting at 0430 and 1630 h in an adjacent herringbone parlor.

The composition of the consumed TMR in nutrients and the offered TMR in ingredients is given in Table 1. The DM contents of the corn silage and the rehydrated and ensiled corn grain were monitored weekly and the TMR was adjusted accordingly. Samples of feeds and orts per cow were obtained daily and frozen for the formation of weekly composites based on equal as-fed amounts of daily samples. Composite samples were dried in a forced-air oven at 55 °C for 72 h and ground to pass a 1-mm mesh screen (Wiley mill; Thomas Scientific, Swedesboro, NJ). The DM concentration was determined by drying at 100 °C for 24 h. The composition of the consumed diet in nutrients was calculated by summation of the intake of nutrients per cow (composition of offered ingredients minus orts on a DM basis) divided by the total DMI for the treatment. The crude protein (**CP**) concentration was determined with a micro Kjeldahl apparatus (AOAC International, 2012), ash by incineration at 550 °C for 8 h, the ash-free neutral detergent fiber (**NDF**) by

Table 1. Diet composition (% of dry matter) and particle size distribution (% of as-fed) on treatments control or BEO

	Control	BEO
Corn silage	47.9	
Tifton hay	3.1	
Finely ground mature corn rehydrated and ensiled	9.1	
Finely ground mature corn	15.4	
Soybean meal	13.5	
Roasted whole soybeans	8.0	
Premix[1]	3.0	
Crude protein	16.5	16.6
Neutral detergent fiber	31.2	31.4
Neutral detergent fiber from forages	26.0	25.7
Ether extract	4.4	4.4
Ash	6.6	6.5
Non-fiber carbohydrates[2]	41.6	41.5
Dry matter, % of as-fed	46.4	46.5
Feed particles >19 mm[3]	9.5 ± 1.55	8.8 ± 1.27
Feed particles 8–19 mm	30.8 ± 5.17	33.1 ± 3.52
Feed particles <8 mm	59.7 ± 6.57	58.1 ± 5.89

[1]44.3% limestone, 21.4% sodium bicarbonate, 10.0% magnesium oxide, 4.3% NaCl, 7.1% urea, and 12.9% minerals and vitamins with 18.5% Ca; 15.0% P; 3.0% Mg; 3.0% S; 240 ppm Co; 3,000 ppm Cu; 8,000 ppm Mn; 12,000 ppm Zn; 90 ppm Se; 180 ppm I; 8,000 KUI/kg Vit. A; 2,000 KUI/kg Vit. D; 50 KUI/kg Vit. E.

[2]NFC = 100 − (Crude protein + Neutral detergent fiber + Ether extract + Ash).

[3]Penn State Particle Separator. Mean and SD of weekly samples.

filtration in porous crucibles with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991), and the ether extract (**EE**) as in AOAC International (2012). The non-fiber carbohydrates (**NFC**) fraction was calculated as follows: 100 – (CP + EE + ash + NDF). The particle size distribution of the fresh TMR was measured on weekly composites of daily samples with the Penn State Particle Separator using the 8- and 19-mm diameter screens and pan.

Variables and Data Collection

Milk yield was recorded daily. Composite milk samples were formed in proportion to the amount produced on each milking on days 6 and 7 of each experimental week. Composite samples were stored in flasks containing 2-bromo-2-nitropropane-1-3-diol under refrigeration until shipping to a commercial laboratory. Milk solids concentration, somatic cell count (**SCC**), and milk urea-N (**MUN**) were measured by mid-infrared analysis (Bentley Instruments Inc., Chaska, MN) at the Laboratory of the Paraná State Holstein Breeders Association (APCBRH, Curitiba, Brazil). Milk energy secretion (**Milk E**; Mcal/d) was calculated as follows: [(0.0929 × % fat) + (0.0547 × % protein) + (0.0395 × % lactose)] × kg of milk (NRC, 2001). The secretion of energy-corrected milk (**ECM**; kg/d) was Milk E/0.70 (assumes 0.70 Mcal/kg of milk with 3.7% fat, 3.2% protein, and 4.6% lactose). The 4% fat-corrected milk (kg/d) was calculated with the Gaines equation (NRC, 2001): 0.4 × kg of milk + 15 × kg of fat.

The BW and BCS were measured at 14-d intervals. The BW was measured after the morning milking during two consecutive days and BCS (1 to 5, thin to fat) was the mean of the same three independent evaluators. The daily gains in BW and BCS were calculated as the slope of the linear regression of BW and BCS over days 0, 14, 28, 42, and 56.

Milk samples obtained on day 56 were frozen at −20 °C for fat isolation and fatty acid profile determination. The daily milk fatty acid secretion (g/d) was calculated assuming that milk fat had 93.3% fatty acids (Glasser et al., 2007). Milk samples were thawed at room temperature and lipid extraction was performed with a mixture of diethyl ether and hexane (AOAC, 1988). The organic phase containing the milk fat was evaporated to dryness at 40 °C under oxygen-free nitrogen. The fatty acid methyl esters were obtained by base-catalyzed transmethylation (Baldin et al., 2013) and quantified using a gas chromatograph (Agilent Technologies: 7820-A, Santa Clara, CA) with a flame-ionization detector equipped with a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm × 0.2 µm film thickness; Varian Inc, Lake Fores, CA). Operating conditions were as provided in the study of Cruz-Hernández et al. (2007).

The total tract apparent digestibilities of DM, organic matter (**OM**), NDF, and non-NDF OM were determined by collection of feces on days 54 to 56. Feces were collected in buckets by one person for every two cows concurrent to defecation during three continuous 8-h sampling periods and weighed. The second and third sampling periods were each delayed by 8 h, thus representing a 24-h collection. Fecal aliquots (equal fresh weight basis) were immediately frozen along the collection period and composite samples were formed per cow. Composite fecal samples were dehydrated for 72 h at 55 °C in a forced air oven. Concentrations of DM, NDF, and ash were determined as previously described.

Viscosity of fecal samples used for the determination of diet digestibility was evaluated according to an adaptation of the methodology of Cannon et al. (2010). Samples of 100 g of fresh feces were diluted in 120 mL of water. The solution was homogenized with a metal spatula for 30 s and filtered through two layers of cheesecloth. The viscosity

of the solution was measured with a rotational viscometer (Model DV-E; Brookfield Engineering Laboratories, Middleboro, MA) at 100 rpm with spindle LV-1 and temperature of 24 °C.

Urine was collected in buckets (one person per two cows) concurrent to urination simultaneously to fecal sampling (days 54 to 56) to estimate the relative ruminal microbial yield based on purine derivative excretion (mmol/d). A 20% sulfuric acid solution (200 mL) was added to 20 L buckets and urine was added to it during the collection period. At the end of the collection period, composite urine samples were diluted 1:5 with a 4% sulfuric acid solution and frozen at −20 °C. Allantoin was analyzed according to Chen and Gomes (1992).

Chewing activity was evaluated on day 49 by visual observation of the buccal activity of each cow at 5-min intervals for 24 h. Buccal activities evaluated were eating, rumination, drinking, and idleness. Eating and rumination per unit of DMI were calculated using the intake of the day in which chewing activity was evaluated. A meal was defined by at least two consecutive 5-min eating events following at least 10 min of idleness or rumination. To generate the meal pattern data, the minimum 10-min intermeal interval was adopted based on the reasoning of Mullins et al. (2012) for a 12-min interval. Meal duration was the ratio between eating time (min/d) and meals per day. Meal size was DMI divided by meals per day. The duration of the first daily meal (conditioned meal) was measured with a chronometer during days 49 to 53. Five evaluators observed the behavior of all cows after offering feed at 0700 h until the last cow finished its first meal.

The proportions of daily intake in periods of the day and particle size sorting behavior were determined on days 51 to 53. The proportions of daily intake in the morning (0700 to 1300 h), afternoon (1300 to 1900 h), and night (1900 to 0700 h) were determined by measuring feed availability per cow at 0700, 1300, and 1900 h and orts at 1300, 1900, and 0700 h. Feed particles sorting behavior was evaluated as the proportion of particles above the 19-mm mesh diameter screen and above and below the 8-mm mesh screen of the Penn State Particle Separator. Particle size distribution was evaluated at 0700 and 1300 h for the offered TMR and at 1300, 1900, and 0700 h for refusals. Feed refusals at 1300 h were mixed with feed offered at 1300 h for measurement of the TMR particle size of each cow. The predicted intake (as-fed basis) of particles on each screen was % TMR retained on screen × kg of TMR consumed. The observed intake of particles was % TMR retained on screen × kg of TMR offered − % orts retained on screen × kg of orts. The selection index was 100× (observed intake/predicted intake). Sorting values below 100 represent selective refusal, above 100 preferential intake, and equal to 100 no selection.

Rectal temperature and respiration rate were measured weekly. Rectal temperature was recorded at 0800, 1400, 2000, and 0200 h with mercury veterinary thermometers (Incoterm, Porto Alegre, Brazil). Respiration rate (breaths/min) was determined at 1400 h as the mean of three consecutive 30 s flank movement countings. Skin surface temperatures of shaved areas of the rump (sacral region) and shoulder blade (scapular region) were measured on days 14, 35, and 56 with an infrared thermometer (Model 88E; HighMed, São Paulo, Brazil) simultaneously to rectal temperature evaluation.

Sweating rate was evaluated on days 14, 35, and 56 at 1400 h. The colorimetric technique used paper disks impregnated with cobalt chloride. Filter paper (Whatman 1, 11 µm porosity) was immersed in a 10% cobalt chloride solution and then oven dried at 100 °C for 12 h. Paper disks (0.5 cm diameter) were cut and dried for 2 min. Three disks were placed on histological slides and fixed with transparent adhesive tape. Slides with disks were immediately placed in a sealed glass containing silica. A 3 × 10 cm rectangular area was shaved 1 d before the measurements on the left flank (paralumbar fossa) of the cows, 20 cm below the dorsal loin. The tape with disks was fixed over the shaved area. The time for each disk to change color from blue violet to light pink was recorded with a chronometer. The mean value was used to calculate the sweating rate (g/m^2 per h) = 38,446.6/T, where T is time in seconds for color change (Schleger and Turner, 1965).

Blood samples were obtained from the coccygeal vessels in vacutainer tubes containing ethylenediaminotetraacetate (EDTA) for the evaluation of plasma urea-N (mg/dL) on day 53 at 0, 1, 2, 3, 6, 9, 12, 15, 18, and 21 h after the morning feeding. Plasma was obtained by centrifugation at 1,800 × g for 10 min and was stored at −20 °C until analysis (Urea 500; Doles Reagentes para Laboratórios Ltda, Goiânia, Brazil). Plasma samples for glucose analysis were obtained in tubes containing EDTA and potassium fluoride 12 h post morning feeding on days 14, 28, and 53 (Glicose Enzimática Líquida; Doles Reagentes para Laboratórios Ltda, Goiânia, Brazil).

Jugular blood acid–base balance was analyzed on days 28 and 53 at 1600 h. Samples were obtained in heparinized tubes and were analyzed with an AGS 22 blood gas analyzer (Drake Eletrônica e

Comércio Ltda, São José do Rio Preto, Brazil) less than 1 h after sampling. Samples were collected from all cows within a 30-min interval, at random within block, and were kept under refrigeration from sampling to analysis.

Reticular fluid samples from all cows were collected on day 56 with a flexible orogastric tube connected to a vacuum pump at random within block. Samples were obtained 12.4 ± 0.68 h after morning feeding, frozen in liquid nitrogen to suppress fermentation, and stored at −20 °C until preparation for analysis of volatile fatty acids by high performance liquid chromatography (Waters Alliance 2998 PDA detector, Milford, MA). The analytical conditions were isocratic mobile phase of 100% aqueous acid solution of phosphoric acid (pH 2.35 to 2.55), oven temperature of 25 ± 5 °C, sample injection volume of 20 µL, run time 25 min, and detector with wavelength at 210 nm. The separation system consisted of reverse-phase C18 ODS 80A (150 × 4.6 mm × 5 µm). Another ruminal sample was mixed to a 36% formaldehyde solution for protozoa counting. The sample was stained and total protozoa were enumerated with an optical microscope in a Neubauer chamber (Warner, 1962).

Sensorial Evaluation of Milk Samples

A triangle test for difference (Lawless and Heymann, 1998) was used to determine if milk consumers would be capable of distinguishing milk of cows fed BEO from milk of cows on control. A sample of raw milk (400 mL/cow) was collected directly from the jars in the milking parlor during the afternoon milking on day 56. Pooled samples for each treatment were formed and kept under refrigeration at 4 °C until the end of the test. The test was performed the next morning and was concluded within 14 h of the start of the milking routine. Sixty-three regular consumers of milk (at least 3×/wk) were recruited among faculty, staff, and students of the University of Lavras. Each assessor received three 50-mL aliquots of milk in glass labeled cups. Two milk samples were from control cows and one sample was from BEO cows. The assessor was asked to identify the odd milk sample after consuming all three samples in random order. Results were expressed as the proportion of correct identification of the odd sample.

Statistical Analysis

Data obtained over time were analyzed with the PROC MIXED of SAS (version 9.3, 2011; SAS Institute Inc., Cary, NC) using a repeated-measures approach. The statistical model contained the covariate effect (measurement of the same variable at the end of the standardization period), the random effect of block (1 to 14), the fixed effects of treatment (control or BEO) and time (day, week, or hours), and the interaction between treatment and time. Cow nested within treatment was defined as the whole plot error. For each variable, the best covariance structure was defined by the Schwarz's Bayesian criteria among first-order autoregressive, compound symmetry, and unstructured. Degrees of freedom were calculated using the Kenward–Roger option. Similar models were used for variables measured once during the experiment and for variables without the covariate adjustment by removing the covariate, time, and its interaction with treatment from the previous model. The proportion of cows with rectal temperature ≥39.2 °C and milk sensorial evaluation data were analyzed with PROC GENMOD using logistic regression for binomial data. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Dry matter intake, lactation performance, and BW change are given in Table 2. The supplementation of BEO reduced DMI by −0.6 kg/d and increased milk yield by +0.7 kg/d, increasing the ratio of milk and ECM to DMI by 0.09 (5.9%) and 0.05 (3.5%), respectively. The increase in feed efficiency in response to BEO was not consistent throughout the experiment, being of larger magnitude (based on within-day treatment differences detected with the SLICE procedure of SAS) from days 2 to 35 (Figure 1). The similarity in feed efficiency after day 35 was associated to the similarity in DMI between treatments (Figure 2). There was a trend for BEO to increase milk fat concentration (+0.11 percentage units), but no effect was detected on the concentrations of protein, lactose, total solids, and MUN. Milk solids yield was not affected by treatment. The linear SCC score, BCS, BW, and BW gain did not differ.

The total tract apparent digestibility of nutrients, ruminal fermentation profile, and plasma glucose and urea-N are given in Table 3. There was a trend for BEO to increase the digestibility of the non-NDF OM and to reduce fecal viscosity and fecal DM concentration. Ruminal microbial yield estimated by the daily allantoin excretion did not differ. The supplementation of BEO reduced the molar proportion of

Table 2. DMI, lactation performance, SCC, MUN, feed efficiency, BCS, and BW on treatments control or BEO

	Control	BEO	SEM	*P*-value[1]
DMI, kg/d	20.1	19.5	0.19	0.05
Milk, kg/d	30.1	30.8	0.20	0.04
4% FCM,[2] kg/d	26.3	26.6	0.23	0.52
ECM,[3] kg/d	27.7	28.0	0.31	0.40
Fat, kg/d	0.943	0.956	0.0294	0.76
Fat, %	3.04	3.15	0.039	0.10
Protein, kg/d	0.961	0.981	0.0240	0.57
Protein, %	3.17	3.19	0.046	0.84
Lactose, kg/d	1.399	1.400	0.0358	0.98
Lactose, %	4.56	4.52	0.023	0.25
Solids, kg/d	3.600	3.633	0.0336	0.48
Solids, %	11.70	11.85	0.082	0.21
Linear SCC,[4] 0 to 9	4.16	4.37	0.168	0.48
MUN, mg/dL	17.0	16.5	0.38	0.37
Milk/DMI	1.53	1.62	0.015	<0.01
ECM/DMI	1.41	1.46	0.014	0.02
BCS, 1 to 5	3.41	3.43	0.060	0.82
BCS gain/d[5]	0.0030	0.0025	0.00143	0.81
BW, kg	651	658	5.4	0.39
BW gain,[5] g/d	350	378	107.2	0.85

[1]$P \le 0.05$ for the effect of time (weeks 1 to 8 or days 1 to 56). $P \ge 0.16$ for the interaction of treatment and time.

[2]4% fat-corrected milk.

[3]Energy-corrected milk.

[4]Equivalency: 4.16 = 224,000 cells/mL and 4.37 = 258,000 cells/mL.

[5]Slope of linear regression of BCS and BW over weeks 0, 2, 4, 6, and 8.

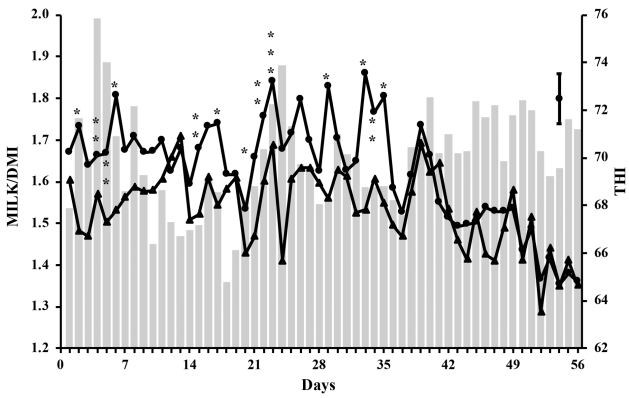

Figure 1. Feed efficiency (milk/dry matter intake) on treatments control (triangles) BEO (circles) and the mean daily THI (Column) during the experiment. $P < 0.01$ for treatment, $P < 0.01$ for day, and $P = 0.46$ for the interaction of treatment and day. SLICE option of SAS: *$P \le 0.05$, **$P \le 0.10$, and ***$P \le 0.15$.

acetate and increased the proportion of propionate, resulting in decreased acetate-to-propionate ratio. The proportion of butyrate did not differ, as well as total protozoa count. Plasma glucose concentration 12 h post-feeding was not affected by treatment, as well as urea-N over time.

The supplementation of BEO had no effect on the fatty acid profile of milk fat ($P \ge 0.13$), except for an increase in the secretion (g/d) of 18:2 *trans*-10, *cis*-12 and in the concentration of 18:0 *iso* fatty acids in milk fat (Table 4). The daily secretion of C 18:0 *iso* also tended to be increased by BEO. The total of short-, medium-, and long-chain fatty acids did not differ.

Chewing behavior is given in Table 5. The durations of eating and rumination behaviors per day and per unit of DMI did not differ. However, BEO induced larger meal size and reduced the number of meals per day. First meal duration tended to be increased by BEO. After being offered the same amount of feed in the morning, cows on BEO had increased proportion of the daily intake from 0700 to 1300 h, which resulted in lower orts as a percentage of the offered TMR at 1300 h. In contrast, a trend for reduced proportion of the daily intake at night on BEO increased orts as a percentage of the offered TMR from 1900 to 0700 h. Feed particle sorting behavior in the morning was also responsive to treatments. From 0700 to 1300 h, BEO induced sorting against long- and medium-length feed particles and selection in favor of small feed particles. There was no treatment effect on feed particles sorting behavior in the afternoon and night.

Body temperature and respiration rate are given in Table 6. The means at 0800, 1400, 2000, and 0200 h for the measurements of rectal and skin surface temperatures at the rump and shoulder blade were unaffected by treatment. The respiration rate measured weekly at 1400 h also did not differ. However, the proportion of rectal temperature measurements ≥39.2 °C was reduced by BEO at 1400 and 2000 h. On day 14, when the mean THI (77.3) and temperature (29.7 °C) were the highest and humidity the lowest (48.6%) among the three sampling-day measurements, BEO increased the sweating rate (Table 7). The treatment effect on sweating rate was not associated to changes in rectal and skin surface temperatures at the moment of the evaluation.

Jugular blood acid–base balance is given in Table 8. The supplementation of BEO increased the partial pressure of O_2, the percentage of oxygen saturation of hemoglobin, and the percentage of oxygen of the total volume of dissolved gases (O_2ct). Blood pH, partial pressure of CO_2, and base excess did not differ.

The capability of BEO to affect milk aroma and flavor was evaluated by the triangular test. The result of the test indicated that 58.7% of assessors (37 correct answers out of 63) were able and 41.3% were unable ($P = 0.05$) to identify an odd milk

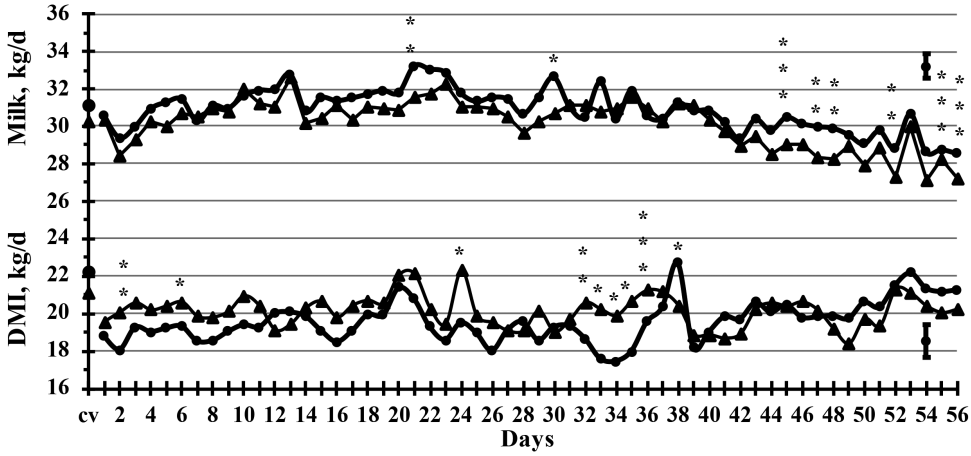

Figure 2. DMI and milk yield (milk) on treatments control (triangles) BEO (circles). DMI: $P = 0.05$ for treatment, $P < 0.01$ for day, and $P = 0.70$ for the interaction of treatment and day. Milk: $P = 0.04$ for treatment, $P < 0.01$ for day, and $P = 0.61$ for the interaction of treatment and day. cv = covariate (DMI $P = 0.40$ and Milk $P = 0.22$). SLICE option of SAS: *$P \leq 0.05$, **$P \leq 0.10$, and ***$P \leq 0.15$.

Table 3. Total tract apparent digestibility (D) of DM, OM, NDF, and non-NDF OM, urinary allantoin excretion and volume, fecal viscosity and DM content, molar volatile fatty acid (VFA) proportions and total protozoa count in ruminal fluid, and plasma glucose and urea-N on treatments control or BEO

	Control	BEO	SEM	*P*-value
DM D, % of intake	68.4	70.6	1.42	0.28
OM D, % of intake	71.1	73.8	1.45	0.20
NDF D, % of intake	46.2	49.3	2.83	0.45
Non-NDF OM D, % of intake	83.5	86.5	1.09	0.07
Allantoin in urine, mmol/d	239	253	22.0	0.67
Urine volume, L/d	19.4	20.3	1.37	0.66
Fecal viscosity, Cp	31.6	24.3	2.44	0.05
Fecal DM, % of fresh	13.7	12.7	0.40	0.05
Acetate, % of total VFA	57.8	51.4	1.52	0.01
Propionate, molar % of total VFA	26.1	31.3	1.43	0.02
Butyrate, molar % of total VFA	16.1	17.3	0.89	0.37
Acetate/propionate	2.3	1.7	0.16	0.03
Protozoa, $\times 10^5$/mL	4.44	5.12	0.624	0.38
Plasma glucose,[1] mg/dL	60.2	58.4	1.63	0.42
Plasma urea-N,[2] mg/dL	14.7	14.7	0.54	0.96

[1]$P = 0.03$ for the effect of day (14, 28, and 53). $P = 0.77$ for the interaction of treatment and day.

[2]$P < 0.01$ for the effect of time (0, 1, 2, 3, 6, 9, 12, 15, 18, and 21 h after morning feeding). $P = 0.16$ for the interaction of treatment and time.

sample coming from cows fed BEO from two samples of control milk.

DISCUSSION

The supplementation of BEO improved feed efficiency, by reducing DMI and increasing milk yield, with a trend for increased milk fat concentration relative to control, without changing BW and BCS. The mechanism for the increase in feed efficiency may have involved change in ruminal fermentation profile. Cows fed BEO had lower acetate-to-propionate ratio than control, suggestive of less methane energy loss. This mode of action of EO on ruminal fermentation has been previously described (Calsamiglia et al., 2007; Benchaar et al., 2008a). Soltan et al. (2018) detected a reduction in the acetate-to-propionate ratio in ruminal fluid of sheep after 3 d of supplementation of the same BEO product used in this experiment.

The positive effect of BEO on feed efficiency was fast acting and was not consistent throughout the experiment. The similarity in DMI induced the similarity in feed efficiency during the last 3 wk of the experiment. During the last third of the experiment, the environment was less variable and high temperature was associated to high humidity, typical of the transition to the rainy-warm season of the year. The THI, temperature, and humidity from days 1 to 36 were [mean ± SD (CV)] 69.4 ± 2.2 (3.2%), 22.3 ± 4.2 (18.8%), and 80.5 ± 14.9 (18.5%) and from days 37 to 56 were 70.9 ± 1.2 (1.7%), 23.4 ± 3.5 (15.9%), and 86.3 ± 3.5 (4.0%), respectively. The effect of BEO on feed efficiency may have interacted with the environment, suggesting better action in cooler and dry environment, although we cannot ascertain this hypothesis. Ruminal adaptation to EO is another possibility for the inconsistency in the response throughout the experiment (Cardozo et al., 2004); however, it is not supported by the occurrence of BEO effects on ruminal and systemic variables measured during week 8.

The absence of BEO effects on total tract digestibility of DM and OM agrees with previous studies with dairy cows that evaluated capsaicin (Oh et al., 2015), oregano leaves rich in carvacrol (Tekippe et al., 2011), and a blend of cinnamaldehyde and

Table 4. Fatty acids in milk fat on treatments control or BEO

	Control	BEO	SEM	*P*-value
	g/100g			
18:1 *trans*-10	0.339	0.433	0.0541	0.25
18:1 *trans*-11	0.780	0.764	0.0346	0.74
18:1 *trans*-10/18:1 *trans* 11	0.444	0.587	0.0702	0.18
18:2 cis-9, *trans*-11	0.461	0.466	0.0273	0.91
18:2 trans-10, *cis*-12	0.0067	0.0080	0.00112	0.44
18:0 *iso*	0.030	0.037	0.0018	0.02
SCFA[1]	26.4	26.5	0.43	0.89
MCFA[2]	28.9	29.1	0.47	0.82
LCFA[3]	41.7	41.5	0.64	0.81
OBCFA[4]	3.0	2.9	0.06	0.87
	g/d			
18:1 *trans*-10	2.685	3.544	0.3732	0.13
18:1 *trans*-11	6.072	6.287	0.5014	0.77
18:1 *trans*-10/18:1 *trans* 11	0.435	0.573	0.0687	0.18
18:2 cis-9, *trans*-11	3.561	3.780	0.2628	0.57
18:2 trans-10, *cis*-12	0.0481	0.0649	0.00482	0.04
18:0 *iso*	0.243	0.308	0.022	0.06
SCFA	206.4	218.3	13.475	0.66
MCFA	228.9	236.9	12.895	0.60
LCFA	324.8	340.4	16.318	0.51
OBCFA	22.5	23.2	1.206	0.69

[1]Short-chain fatty acids: 4:0, 6:0, 8:0, 10:0, 10:1 *cis*-9, 12:0, 12:1 *cis*-9 + 13:0, 14:0, 14:1 *cis*-9.

[2]Medium-chain fatty acids: 16:0, 16:1 *trans*-9 + 17:0 *iso*, 16:1 *trans*-12, 16:1 *cis*-9 + 17:0 *anteiso*.

[3]Long-chain fatty acids: 18:0, 18:1 *trans*-4, 18:1 *trans*-5, 18:1 *trans*-6 to 8, 18:1 *trans*-9, 18:1 *trans*-10, 18:1 *trans*-11, 18:1 *trans*-12, 18:1 *trans*-13 and 14, 18:1 *cis*-9, 18:1 *cis*-11, 18:1 *cis*-12, 18:1 *trans*-16, 18:1 *cis*-15 + 19:0, 18:2 *trans*-9, *trans*-12, 18:2 *cis*-9, *trans*-12, 18:2 *cis*-12, *trans*-9, 18:2 *cis*-9, *trans*-11, 18:2 *trans*-9, *cis*-11–12, 18:2 *trans*-10, *cis*-12, 18:2 *n*-6, 18:3 *n*-6, 18:3 *n*-3, 20:0, 20:1 *cis*-11, 20:2 *n*-6, 20:3 *n*-6, 20:4 *n*-6, 20:4 *n*-3, 20:5 *n*-3, 22:0, 22:5 *n*-3, 24:0.

[4]Odd- and branched-chain fatty acids: 5:0, 7:0, 9:0, 11:0, 14:0 *iso*, 15:0 *iso*,15:0 *anteiso*, 16:0 *iso*, 17:0, 18:0 *iso*, 17:1 *cis*-9, 21:0 23:0.

Table 5. Rumination, eating, and sorting behavior on treatments control or BEO

	Control	BEO	SEM	*P*-value
Eating, min/d	290	283	9.3	0.78
Rumination, min/d	510	503	17.5	0.59
Chewing,[1] min/d	800	786	22.8	0.67
Eating, min/kg DMI	13.4	14.8	0.82	0.23
Rumination, min/kg DMI	23.3	26.5	1.60	0.20
Chewing, min/kg DMI	36.8	41.3	2.33	0.19
Meal size, kg of DM/meal	1.5	1.7	0.08	<0.01
First meal duration, min	44	53	3.4	0.08
Meal duration, min	21	23	0.97	0.15
Meals/d	13.7	11.9	0.61	0.05
0700 to 1300 h, % of daily intake	30.6	36.6	1.59	0.02
1300 to 1900 h, % of daily intake	47.3	45.8	1.27	0.42
1900 to 0700 h, % of daily intake	22.1	17.6	1.56	0.06
Observed/Predicted,[2] %				
0700 to 1300 h				
>19 mm[3]	112	69	11.5	0.03
>8 mm and <19 mm[3]	93	66	3.1	<0.01
<8 mm[3]	102	117	2.5	<0.01
1300 to 1900 h				
>19 mm	96	90	6.1	0.47
>8 mm and <19 mm	86	89	4.4	0.55
<8 mm	106	107	2.6	0.83
1900 to 0700 h				
>19 mm	95	127	17.0	0.21
>8 mm and <19 mm	93	92	2.60	0.81
<8 mm	106	129	14.7	0.30
Orts, % of offered (as-fed basis)				
Orts 0700 to 1300 h	53.2	44.3	2.05	0.03
Orts 1300 to 1900 h	37.6	39.2	3.27	0.86
Orts 1900 to 0700 h	22.9	40.5	6.91	0.04
Daily orts,[4]	6.2	10.6	5.75	0.33

[1]Eating + rumination.

[2]<100 = sorting against, >100 = sorting in favor, 100 = no selection.

[3]Sieves of the Penn State Particle Separator. Long-length particles (>19 mm), medium-length particles (>8 mm and <19 mm), and short-length particles (<8 mm).

[4]Daily orts = (Orts 0700 h/Offered TMR per day) × 100.

eugenol (Tager and Krause, 2011). A trend was observed for increased digestibility of the non-NDF OM with BEO, and non-statistical increases were also observed for OM and NDF digestibilities. Starch was the major NFC source in the diet, suggesting that the reduction in ruminal acetate-to-propionate ratio may have been the result of increased ruminal starch degradation. Unfortunately, ruminal or total tract starch digestibility was not measured in this experiment.

Fecal viscosity and DM concentration were positively correlated (r = 0.66, P = 0.03) and were both reduced by BEO. The effect of BEO on fecal viscosity may have involved the effect on water ingestion. Holstein heifers fed a blend of eugenol and cinnamaldehyde had reduced water ingestion and capsicum oil increased water ingestion (Cardozo et al., 2006). Fecal viscosity is a poorly studied parameter in ruminant nutrition and its value as a variable in nutrition studies requires further evaluation.

The supplementation of BEO tended to increase first meal duration, reduced meal frequency, and increased meal size. Larger meal size may increase the post-meal decay in ruminal pH (Dado and Allen, 1993). The supplementation of BEO also increased the proportion of daily intake in the morning and induced selective sorting in favor of short feed particles and against long particles, also capable of decreasing post-feeding ruminal pH (DeVries et al., 2008). Tager and Krause (2011) observed that a blend of cinnamaldehyde and eugenol or capsicum supplemented to lactating cows shortened the length of the

Table 6. Temperatures of skin surface and rectal, respiration rate, and the proportion of cows with rectal temperature ≥39.2 °C at 0800, 1400, 2000, and 0200 h on treatments control or BEO

	Control	BEO	SEM	*P*-value[1]
Skin surface, °C				
Rump				
0800 h	33.2	33.4	0.31	0.63
1400 h	33.9	33.8	0.23	0.74
2000 h	34.5	34.5	0.13	0.94
0200 h	33.0	33.3	0.26	0.50
Shoulder				
0800 h	33.2	33.0	0.25	0.65
1400 h	33.9	33.7	0.25	0.61
2000 h	34.0	34.5	0.13	0.13
0200 h	33.3	33.2	0.20	0.76
Rectal, °C				
0800 h	38.4	38.3	0.04	0.45
1400 h	38.8	38.7	0.05	0.23
2000 h	38.8	38.8	0.05	0.79
0200 h	38.7	38.6	0.07	0.80
Respiration rate, breaths/min				
1400 h	50	48	1.56	0.32
% of cows ≥39.2 °C[2]				
0800 h	4.46	0.89		0.13
1400 h	28.5	17.8		0.05
2000 h	34.8	23.2		0.05
0200 h	20.5	17.8		0.61

[1]$P < 0.01$ for the effect of week (1 to 8) for rectal temperature and respiration rate, and day (14, 35, and 56) for skin surface temperature. $P \geq 0.17$ for the interaction of treatment and time.

[2]Observations/treatment = 112 (14 cows during 8 wk). GENMOD procedure of SAS using logistic regression for binomial data.

Table 7. Sweating rate at 1400 h and the temperatures of skin surface and rectal during the measurement on treatments control or BEO

	Control	BEO	SEM	*P*-value
Day 14				
Sweating rate, g/m² per h	160	221	21.6	0.05
Rectal temperature, °C	38.7	38.7	0.09	0.91
Skin surface temperature shoulder, °C	34.0	33.8	0.23	0.53
Skin surface temperature rump, °C	33.7	33.8	0.27	0.78
Day 35				
Sweating rate, g/m² per h	138	170	15.1	0.15
Rectal temperature, °C	38.6	38.5	0.14	0.66
Skin surface temperature shoulder, °C	33.2	32.8	0.33	0.24
Skin surface temperature rump, °C	32.3	33.0	0.40	0.21
Day 56				
Sweating rate, g/m² per h	424	441	59.7	0.84
Rectal temperature, °C	38.9	38.8	0.08	0.63
Skin surface temperature shoulder, °C	35.5	34.9	0.39	0.55
Skin surface temperature rump, °C	35.0	35.1	0.36	0.87

[1]Mean environmental temperature, humidity, and THI of the day of measurements. Day 14: 29.7 °C, 48.6%, and 77.3; day 35: 27.3 °C, 83.8%, and 72.4; day 56: 26.1 °C, 82.6%, and 75.5.

Table 8. Jugular blood acid–base balance at 1600 h on treatments control or BEO

	Control	BEO	SEM	*P*-value[1]
pH	7.48	7.46	0.011	0.46
pCO_2,[2] mm Hg	40.94	41.60	0.667	0.50
pO_2,[3] mm Hg	28.49	36.61	1.689	<0.01
HCO_3^-, mmol/L	30.41	30.56	0.490	0.83
Total CO_2, mmol/L	31.69	31.85	0.501	0.82
Base excess, mmol/L	7.01	7.07	0.479	0.93
$SatO_2$,[4] %	56.06	68.19	1.980	<0.01
O_2ct,[5] %	12.76	15.27	0.444	<0.01

[1]$P < 0.01$ for the effect of day (28 and 53). $P \geq 0.16$ for the interaction of treatment and day.

[2]Partial pressure of CO_2.

[3]Partial pressure of O_2.

[4]Oxygen saturation, % of oxygen based on total hemoglobin saturation capacity.

[5]Oxygen content, % of oxygen related to the total volume of dissolved gases.

first meal without affecting DMI, lactation performance, ruminal fermentation profile, and diet digestibility. Those authors suggested that EO changed diet palatability. Various factors, such as product dosage, type of EO, and form of supplementation, may determine how EO affect eating behavior and a defined pattern of response does not seem to be predictable for this class of feed additive.

The BEO reduced the proportion of cows with rectal temperature ≥39.2 °C, suggestive of heat stress alleviation (Rhoads et al., 2009). Cows were subjected to THI greater or equal to 68 for 68.6% of the experiment. Cows on BEO had higher sweating rates than control only during a hot and low-humidity day. Evaporative heat losses are favored by low relative humidity (Curtis, 1983). Capsaicin can act on body thermoregulation by stimulation of the transient receptor potential vanilloid 1 (TRPV1) in the oral cavity (Inoue et al., 2007). Eugenol is also able to bind to capsaicin receptors (Xu et al., 2006). Capsaicin activates warm-sensitive neurons and inhibits cold-sensitive neurons via TRPV1 receptor on the preoptic-anterior area of the hypothalamus and can act directly on warm-sensitive neurons (Caterina, 2007). Lee et al. (2000) reported a decrease in body temperature (37.1 vs. 36.8 °C) of rats treated with 5 mg/kg of capsaicin via intraperitoneal injection. Capsaicin (Starr et al., 2008), carvacrol (Murphy et al., 2016),

cinnamaldehyde (Yanaga et al., 2006), and eugenol (Damiani et al., 2003) have also been shown to induce vasodilation. Such effects are usually dose dependent and can be triggered by different mechanisms (e.g., calcium influx, cell hyperpolarization, TRPV receptors, or nitric oxide). A relief in heat stress induced by BEO might reduce energetic expenditures associated with heat stress remediation, increasing the energetic efficiency of the animal by a post-absorptive mechanism, in addition to the already discussed gain in the energetic efficiency of the ruminal fermentation as the result of reduced acetate-to-propionate ratio. At the same respiration rate, cows on BEO had higher jugular blood oxygenation than control. There are reports of increased blood hemoglobin concentration in cows fed capsicum oleoresin (Oh et al., 2013, 2015).

Cows fed BEO secreted (g/d) more conjugated linoleic acid (CLA) (18:2 *trans*-10, *cis*-12), suggesting that EO affected ruminal polyunsaturated fatty acids (PUFA) biohydrogenation. The diet had coarsely ground whole roasted soybeans as a linoleic acid-rich fat source, corn silage as the major forage, and corn grain (ensiled and ground) as additional starch sources, all capable of reducing milk fat secretion, as observed (3.1% fat in milk). The only feed additives in the basal diet were a buffer and an alkalizer. The increased secretion of 18:2 *trans*-10, *cis*-12 on BEO was associated to a trend for increased milk fat % with no effect on fat yield, which we would not expect for this CLA known to inhibit mammary gland fat synthesis (Baumgard et al., 2000). The similarity in the proportions of short-, medium, and long-chain fatty acids in milk fat also suggests that fat synthesis was not affected by 18:2 *trans*-10, *cis*-12. The increased secretion of 18:2 *trans*-10, *cis*-12 suggests that BEO probably favored this PUFA biohydrogenation route in the rumen. Lourenço et al. (2008) observed that cinnamaydehyde increased the proportion of 18:1 *trans*-10 and 18:2 *trans*-10, *cis*-12 fatty acids in a continuous culture fermenter. Ruminal biohydrogenating bacteria were affected by EO in vitro (Patra and Yu, 2015). The positive effect of BEO on 18:0 *iso* in milk fat may have been the result of improved PUFA biohydrogenation in the rumen, although we cannot verify this hypothesis.

Based on the sensorial test performed, refrigerated raw milk from cows fed BEO was correctly identified by the majority of regular consumers of milk. However, the test evaluated the capacity of identifying an odd milk sample, being not a measure of preference or of the perceived aroma and flavor of the samples. Milk from BEO cows had similar composition to control milk during the sensorial evaluation. The analyzed composition of the BEO and control milk was 3.10 vs. 3.04% fat, 3.27 vs. 3.24% protein, and 4.44 vs. 4.55% lactose, respectively. The presence of EO in milk may explain the perception of the odd sample. The transfer of volatile EO to milk through respiratory and gastrointestinal exposure has been demonstrated (Lejonklev et al., 2013). Trained sensory assessors detected that six sensorial attributes differed in milk from cows fed with terpenes, the EO resulted in milk with fresher aroma and lower stored aroma and flavor than control (Lejonklev et al., 2016). Essential oils may be a strategy for the introduction of organoleptic and nutritional properties in dairy products.

CONCLUSIONS

The supplementation of lactating cows with BEO (capsaicin, carvacrol, cinnamaldehyde, and eugenol) reduced DMI and increased milk yield. The BEO reduced the acetate-to-propionate ratio in ruminal fluid, altered eating behavior, lowered the frequency of high rectal temperature, and increased blood oxygenation. The BEO increased feed efficiency and can potentially add aroma and flavor to dairy products in a natural manner.

ACKNOWLEDGMENT

R.B.S, M.N.P., R.C.A., W.R.S, and R.A.N.P. are grateful to Fapemig (Minas Gerais Foundation for Research Support, Belo Horizonte, Brazil) and CNPq (National Research Council, Brasilia, Brazil) for the grants, to GRASP Indústria e Comércio Ltda (Curitiba, Brazil) for partially funding the project, to the members of Grupo do Leite of the University of Lavras for the help in data collection and animal care, and to Dr. Marco Antonio Sundfeld da Gama (Embrapa CNPGL, Brazilian Agricultural Research Corporation, Juiz de Fora, Brazil) for the Gas Liquid Chromatography analysis.

Conflict of interest statement: None declared.

LITERATURE CITED

AOAC International. 1988. Official methods of analysis fat in milk. JAOAC. 71:898.

AOAC International. 2012. Official methods of analysis. 19th ed. Arlington, VA: Association of Official Analytical Chemists; p. 409.

Baldin, M., M. A. Gama, R. Dresch, K. J. Harvatine, and D. E. Oliveira. 2013. A rumen unprotected conjugated linoleic acid supplement inhibits milk fat synthesis and improves energy balance in lactating goats. J. Anim. Sci. 91:3305–3314. doi:10.2527/jas.2012-5766

Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Saebø, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am.

J. Physiol. Regul. Integr. Comp. Physiol. 278:R179–R184. doi:10.1152/ajpregu.2000.278.1.R179

Benchaar, C., S. Calsamiglia, A. V. Chaves, G. R. Fraser, D. Colombatto, T. A. McAllister, and K. A. Beauchemin. 2008a. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol. 145:209–228. doi:10.1016/j.anifeedsci.2007.04.014

Benchaar, C., T. A. McAllister, and P. Y. Chouinard. 2008b. Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or Yucca schidigera saponin extracts. J. Dairy Sci. 91:4765–4777. doi:10.3168/jds.2008-1338

Calsamiglia, S., M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret. 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. 90:2580–2595. doi:10.3168/jds.2006-644

Cannon, S. J., G. C. Fahey, Jr, M. R. Murphy, C. L. Dikeman, B. L. Miller, and J. K. Drackley. 2010. Inclusion of psyllium in milk replacer for neonatal calves. 1. Effects on growth, digesta viscosity, rate of passage, nutrient digestibilities, and metabolites in blood. J. Dairy Sci. 93:3652–3660. doi:10.3168/jds.2009-2731

Cardozo, P. W., S. Calsamiglia, A. Ferret, and C. Kamel. 2004. Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. J. Anim. Sci. 82:3230–3236. doi:10.2527/2004.82113230x

Cardozo, P. W., S. Calsamiglia, A. Ferret, and C. Kamel. 2006. Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. J. Anim. Sci. 84:2801–2808. doi:10.2527/jas.2005-593

Caterina, M. J. 2007. Transient receptor potential ion channels as participants in thermosensation and thermoregulation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292:R64–R76. doi:10.1152/ajpregu.00446.2006

Chen, X. B., and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: an overview of technical details. Aberdeen (UK): Int. Feed Res. Unit, Occasional Publ. Rowett Research Institute.

Cruz-Hernandez, C., J. K. Kramer, J. J. Kennelly, D. R. Glimm, B. M. Sorensen, E. K. Okine, L. A. Goonewardene, and R. J. Weselake. 2007. Evaluating the conjugated linoleic acid and *trans* 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. J. Dairy Sci. 90:3786–3801. doi:10.3168/jds.2006-698

Curtis, S. E. 1983. Environmental management in animal agriculture. Ames (IO): Iowa State Press.

Dado, R. G., and M. S. Allen. 1993. Relationship between eating behavior and ruminal pH for dairy cows consuming low or high fiber diets. In: Abstr. Conf. Rumen Function. Ithaca (NY): Cornell University; p. 18.

Damiani, C. E., L. V. Rossoni, and D. V. Vassallo. 2003. Vasorelaxant effects of eugenol on rat thoracic aorta. Vascul. Pharmacol. 40:59–66. doi:10.1016/s1537-1891(02)00311-7

Devries, T. J., F. Dohme, and K. A. Beauchemin. 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: feed sorting. J. Dairy Sci. 91:3958–3967. doi:10.3168/jds.2008-1347

García-García, R., A. López-Malo, and E. Palou. 2011. Bactericidal action of binary and ternary mixtures of carvacrol, thymol, and eugenol against *Listeria innocua*. J. Food Sci. 76:M95–100. doi:10.1111/j.1750-3841.2010.02005.x

Glasser, F., M. Doreau, A. Ferlay, and Y. Chilliard. 2007. Technical note: estimation of milk fatty acid yield from milk fat data. J. Dairy Sci. 90:2302–2304. doi:10.3168/jds.2006-870

Hashemzadeh-Cigari, F., M. Khorvash, G. R. Ghorbani, M. Kadivar, A. Riasi, and Q. Zebeli. 2014. Effects of supplementation with a phytobiotics-rich herbal mixture on performance, udder health, and metabolic status of Holstein cows with various levels of milk somatic cell counts. J. Dairy Sci. 97:7487–7497. doi:10.3168/jds.2014-7989

Hristov, A. N., C. Lee, T. Cassidy, K. Heyler, J. A. Tekippe, G. A. Varga, B. Corl, and R. C. Brandt. 2013. Effect of *Origanum vulgare* L. leaves on rumen fermentation, production, and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 96:1189–1202. doi:10.3168/jds.2012-5975

Inoue, N., Y. Matsunaga, H. Satoh, and M. Takahashi. 2007. Enhanced energy expenditure and fat oxidation in humans with high BMI scores by the ingestion of novel and non-pungent capsaicin analogues (capsinoids). Biosci. Biotechnol. Biochem. 71:380–389. doi:10.1271/bbb.60341

Khiaosa-ard, R., and Q. Zebeli. 2013. Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants. J. Anim. Sci. 91:1819–1830. doi:10.2527/jas.2012-5691

Klevenhusen, F., A. Muro-Reyes, R. Khiaosa-ard, B. U. Metzler-Zebeli, and Q. Zebeli. 2012. A meta-analysis of effects of chemical composition of incubated diet and bioactive compounds on *in vitro* ruminal fermentation. Anim. Feed Sci. Technol. 176:61–69. doi:10.1016/j.anifeedsci.2012.07.008

Lawless, H. T., and H. Heymann. 1998. Sensory evaluation of food: principles and practices. New York (NY): Chapman & Hall; p. 173–207.

Lee, T. H., J. W. Lee, T. Osaka, A. Kobayashi, Y. Namba, S. Inoue, and S. Kimura. 2000. Lack of integrative control of body temperature after capsaicin administration. Korean J. Intern. Med. 15:103–108. doi:10.3904/kjim.2000.15.2.103

Lejonklev, J., U. Kidmose, S. Jensen, M. A. Petersen, A. L. F. Helwing, G. Mortensen, M. R. Weisbjerg, and M. K. Larsen. 2016. Short communication: effect of oregano and caraway essential oils on the production and flavor of cow milk. J. Dairy Sci. 99:7898–7903. doi:10.3168/jds.2016-10910

Lejonklev, J., M. M. Løkke, M. K. Larsen, G. Mortensen, M. A. Petersen, and M. R. Weisbjerg. 2013. Transfer of terpenes from essential oils into cow milk. J. Dairy Sci. 96:4235–4241. doi:10.3168/jds.2012-6502

Lourenço, M., P. W. Cardozo, S. Calsamiglia, and V. Fievez. 2008. Effects of saponins, quercetin, eugenol, and cinnamaldehyde on fatty acid biohydrogenation of forage polyunsaturated fatty acids in dual-flow continuous culture fermenters. J. Anim. Sci. 86:3045–3053. doi:10.2527/jas.2007-0708

Mullins, C. R., L. K. Mamedova, M. J. Brouk, C. E. Moore, H. B. Green, K. L. Perfield, J. F. Smith, J. P. Harner, and B. J. Bradford. 2012. Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. J. Dairy Sci. 95:1323–1336. doi:10.3168/jds.2011-4744

Murphy, T. V., A. Kanagarajah, S. Toemoe, P. P. Bertrand, T. H. Grayson, F. C. Britton, L. Leader, S. Senadheera, and S. L. Sandow. 2016. TRPV3 expression and vasodilator function in isolated uterine radial arteries from non-pregnant and pregnant rats. Vascular Pharmacology. 83:66–77. doi:10.1016/j.vph.2016.04.004

NRC. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Washington (DC): National Academy Press.

Oh, J., F. Giallongo, T. Frederick, J. Pate, S. Walusimbi, R. J. Elias, E. H. Wall, D. Bravo, and A. N. Hristov. 2015. Effects of dietary *Capsicum* oleoresin on productivity and immune responses in lactating dairy cows. J. Dairy Sci. 98:6327–6339. doi:10.3168/jds.2014-9294

Oh, J., A. N. Hristov, C. Lee, T. Cassidy, K. Heyler, G. A. Varga, J. Pate, S. Walusimbi, E. Brzezicka, K. Toyokawa, et al. 2013. Immune and production responses of dairy cows to postruminal supplementation with phytonutrients. J. Dairy Sci. 96:7830–7843. doi:10.3168/jds.2013-7089

Patra, A. K., and Z. Yu. 2015. Essential oils affect populations of some rumen bacteria in vitro as revealed by microarray (RumenBactArray) analysis. Front. Microbiol. 6:297. doi:10.3389/fmicb.2015.00297

Rhoads, M. L., R. P. Rhoads, M. J. VanBaale, R. J. Collier, S. R. Sanders, W. J. Weber, B. A. Crooker, and L. H. Baumgard. 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J. Dairy Sci. 92:1986–1997. doi:10.3168/jds.2008-1641

Santos, M. B., P. H. Robinson, P. Williams, and R. Losa. 2010. Effects of addition of an essential oil complex to the diet of lactating dairy cows on whole tract digestion of nutrients and productive performance. Anim. Feed Sci. Technol. 157:64–71. doi:10.1016/j.anifeedsci.2010.02.001

Schleger, A. V., and H. G. Turner. 1965. Sweating rates of cattle in the field and their reaction to diurnal and seasonal changes. Aust. J. Agric. Res. 16:92–106. doi:10.1071/AR9650092

Soltan, Y. A., A. S. Natel, R. C. Araujo, A. S. Morsy, and A. I. Abdalla. 2018. Progressive adaptation of sheep to a microencapsulated blend of essential oils: ruminal fermentation, methane emission, nutrient digestibility, and microbial protein synthesis. Anim. Feed. Sci. Technol. 237:8–18. doi:10.1016/j.anifeedsci.2018.01.004

Spanghero, M., P. H. Robinson, C. Zanfi, and E. Fabbro. 2009. Effect of increasing doses of a microencapsulated blend of essential oils on performance of lactating primiparous dairy cows. Anim. Feed Sci. Technol. 153:153–157. doi:10.1016/j.anifeedsci.2009.06.004

Starr, A., R. Graepel, J. Keeble, S. Schmidhuber, N. Clark, A. Grant, A. M. Shah, and S. D. Brain. 2008. A reactive oxygen species-mediated component in neurogenic vasodilatation. Cardiovasc. Res. 78:139–147. doi:10.1093/cvr/cvn012

Suganthi, R. U., and S. Manpal. 2013. Biological and pharmacological actions of carvacrol and its effects on poultry: an updated review. World J. Pharm. Sci. 5:3581–3595.

Tager, L. R., and K. M. Krause. 2011. Effects of essential oils on rumen fermentation, milk production, and feeding behavior in lactating dairy cows. J. Dairy Sci. 94:2455–2464. doi:10.3168/jds.2010-3505

Tassoul, M. D., and R. D. Shaver. 2009. Effect of a mixture of supplemental dietary plant essential oils on performance of periparturient and early lactation dairy cows. J. Dairy Sci. 92:1734–1740. doi:10.3168/jds.2008-1760

Tekippe, J. A., A. N. Hristov, K. S. Heyler, T. W. Cassidy, V. D. Zheljazkov, J. F. Ferreira, S. K. Karnati, and G. A. Varga. 2011. Rumen fermentation and production effects of *Origanum vulgare* L. leaves in lactating dairy cows. J. Dairy Sci. 94:5065–5079. doi:10.3168/jds.2010-4095

Tekippe, J. A., R. Tacoma, A. N. Hristov, C. Lee, J. Oh, K. S. Heyler, T. W. Cassidy, G. A. Varga, and D. Bravo. 2013. Effect of essential oils on ruminal fermentation and lactation performance of dairy cows. J. Dairy Sci. 96:7892–7903. doi:10.3168/jds.2013-7128

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Warner, A. C. 1962. Enumeration of rumen micro-organisms. J. Gen. Microbiol. 28:119–128. doi:10.1099/00221287-28-1-119

Xu, H., M. Delling, J. C. Jun, and D. E. Clapham. 2006. Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat. Neurosci. 9:628–635. doi:10.1038/nn1692

Yanaga, A., H. Goto, T. Nakagawa, H. Hikiami, N. Shibahara, and Y. Shimada. 2006. Cinnamaldehyde induces endothelium-dependent and -independent vasorelaxant action on isolated rat aorta. Biol. Pharm. Bull. 29:2415–2418. doi:10.1248/bpb.29.2415

Yousef, M. K. 1985. Stress physiology in livestock, 1st ed. Boca Raton (FL): CRC Press Inc.