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Effects of functional oils on ruminal fermentation, rectal temperature, and performance of dairy cows under high temperature humidity index environment

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ABSTRACT

The hypothesis of the current study was that functional oils (FO) could replace monensin (MON) in diets while improving thermal status and performance of dairy cows. This study was designed to evaluate the effects of a blend of FO and MON on nutrient intake and total tract digestion, ruminal fermentation, glucose and urea concentrations in blood, heart rate, rectal temperature, and milk yield and composition of dairy cows under heat stress in Brazil. Thirty-six Holstein cows [201 ± 63 days in milk, 599 ± 78 kg of body weight, and 28.7 ± 3.92 kg/d of milk yield (mean ± SD)] were used in a randomized complete block design experiment that lasted six weeks (one week for covariate adjustments and five weeks for treatment effect evaluation). Cows within blocks were assigned individually to one of the following treatments: 1) control (CON), basal diet without feed additives; 2) functional oils (FO), basal diet added 500 mg/kg DM of a commercial blend of cashew nut shell liquid and castor oil; and 3) monensin (MON), basal diet added 22 mg/kg DM of sodium monensin. Additives were provided mixed into the concentrate and total mixed ration (TMR) was fed twice daily. The experimental barn daily temperature, air relativity humidity, and temperature humidity index were 25.0 ± 0.25 °C, 81.7 ± 1.17%, and 74.6 ± 0.28, respectively (mean ± SE), suggesting that heat stress likely impacted cows. Cows fed MON had lower ($P = 0.012$) dry matter intake despite treatments have not affected total tract digestibility of nutrients and ruminal fermentation (pH, NH_3-N, and VFA concentration). Both MON and FO increased ($P = 0.006$) serum urea concentration without affecting serum glucose levels. Treatments had no effect on heart rate, respiration rate, and rectal temperature. Although treatments did not affect milk yield and fat-corrected milk, cows fed FO had a greater ($P = 0.021$) milk fat content than those fed MON. Cows fed FO exhibited the highest value of milk fat content (34.6, 32.5, and 36.5 g/kg for CON, MON, and FO, respectively). This study did not show evidence that treatments can decrease body temperature, but FO may replace MON in diets to

Abbreviations: ADF, acid detergent fiber; BCFA, branched-chain fatty acids; C2C3 ratio, acetate to propionate ratio; BW, body weight; CNSL, cashew nut shell liquid; CO, castor oil; CON, control; CP, crude protein; RH, relative humidity; DM, dry matter; DMI, dry matter intake; EE, ether extract; FCM, fat-corrected milk; FO, function oil; iNDF, indigestible neutral detergent fiber; MON, monensin; NDF, neutral detergent fiber; NEL, net energy of lactation; SD, standard deviation; THI, temperature humidity index; TMR, total mixed ration; VFA, volatile fatty acids

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maintain milk fat concentration without affecting dry matter intake and milk yield of cows under heat stress.

1. Introduction

Heat stress still a costly issue for the dairy industry despite the advances in cooling systems and management practices. St-Pierre et al. (2003) estimated that heat stress imposes annual costs for the U.S. dairy industry around $848.4 million when optimum mitigation strategy is adopted whereas costs can reach US$1.5 billion dollar when no strategies are adopted to minimize the impacts of heat stress. These costs are related to metabolic/health disorders, decreases in milk yield and milk quality, and impairments in reproductive performance of cows impacted by heat stress (Collier et al., 2017). Above the thermoneutral zone, cows experience changes in respiration rate and blood concentration of metabolites and hormones, and may present hepatic dysfunctions (Omar et al., 1996; Bernabucci et al., 2010; Weelock et al., 2010; Baumgard et al., 2011) which result in decreased milk output and milk solids content (Kadzere et al., 2002).

Ionophore antibiotics [e.g., monensin (MON)] have been used over the last decades to improve animal performance by increasing the rate of propionate production, lowering methane production, and reducing ruminal ammonia accumulation (reviewed by McGuffey et al., 2001). However, evidence suggests that MON increases the rectal temperature and respiration rates of cows either in thermal neutral or under heat stress conditions (Baumgard et al., 2011). In addition, the use of ionophore antibiotics as feed additives has been reduced worldwide, turning. Ionophores can still be used as feed additives in some places.

Recently, authors have reported that a blend of plant extracts (named “functional oils”) improves the performance of lactating cows, presumably by increasing ruminal propionate concentration (Ferreira de Jesus et al., 2016). The functional oils (FO) used in the latter study consisted of a blend of cashew nut shell liquid (CNSL) and castor oil (CO), which cannot be classified as essential oils since they are not derived from essences or spices. Instead, these oils are referred as FO because they exhibit biological properties beyond their nutritional value (Murakami et al., 2014; Bess et al., 2012).

Cashew nut shell liquid is a co-product of the cashew nut (*Anacardium accidentale*) industry composed of resorcinolic and alkylphenolic oil (Murakami et al., 2014), with anacardic acid (3-*n*-pentadecylsalicylic acid), cardanol (3-*n*-pentadecylphenol), cardol (5-*n*-pentadecylresorcinol), and methylcardol (2-methyl-5-*n*-pentadecylresorcinol) as bioactive compounds (Murakami et al., 2014;

Table 1
Ingredient and chemical composition of experimental diet (g/kg DM, otherwise stated).

Item	Experimental basal diet
Ingredient	
Corn Silage	500
Ground corn	196
Soybean meal	129
Whole raw soybean	60.5
Citrus pulp	59.9
Bypass soybean meal[a]	21.2
Mineral and vitamin premix[b]	15.2
Limestone	3.31
Sodium bicarbonate	9.05
Urea	2.70
Salt	2.46
Chemical composition	
Dry matter (g/kg as-fed)	458
Organic matter	921
Non-fiber carbohydrate[c]	423
Neutral detergent fiber (NDF)	290
Acid detergent fiber	193
Indigestible NDF	101
Crude protein	164
Ash	79.4
Ether extract	43.0
Lignin	24.3
NEL_{3x}[d]	1.65

[a] Soypass Brazil® (Cargill, Uberlândia, Brazil).
[b] Each kg of premix contained: 205 g Ca, 60 g P, 35 g K, 70 g Na, 20 g S, 20 g Mg, 2,500 mg Zn, 1,600 mg Mn, 700 mg of Cu, 700 mg Fe, 40 mg I, 19 mg Se, 10 mg Cr, 200,000 IU vitamin A, 50,000 IU vitamin D, and 1500 IU vitamin E.
[c] Calculated according to Hall, 2000.
[d] NEL_{3x}: Net energy of lactation at 3 times of the maintenance intake level, according NRC (2001) equations.

Voirin et al., 2014). Cashew nut shell liquid has decreased methanogenesis, increased the ruminal proportion of propionate in non-lactating cows (Shinkai et al., 2012), and tended to decrease rumen methane emission and marginally increased total tract neutral detergent fiber (NDF) digestibility in lactating cows (Branco et al., 2015). Castor oil (CO), obtained from castor seed (*ricinus communis*) pressing, has approximately 90% of ricinoleic acid as entire fatty acid chain composition (Vieira et al., 2001). It can modulate the ruminal bacterial population in beef cattle (Zotti et al., 2017) and may improve the performance of lactating cows (Gandra et al., 2014). CNSL and CO are biologically active presenting gastroprotective (Hamad and Mubofu, 2015), anti-inflammatory (Vieira et al., 2000), and antioxidant properties (Trevisan et al., 2006). These properties may be more beneficial in cows impacted by heat stress since hyperthermia alone promotes the production of reactive oxygen and nitrogen species that damage cell membranes and tight junctions of intestinal epithelium (Bernabucci et al., 2002; Lambert et al., 2002).

Although the effects of the blend of CNSL and CO on performance of dairy cows were described by Ferreira de Jesus et al. (2016), these compounds were not explored in cows impacted by heat stress. We hypothesized that FO could replace MON in diets of dairy cows while improving thermal status and performance. The objective of this study was to determine the effects of a blend of FO or MON on total tract digestibility of nutrients, ruminal fermentation, levels of glucose and urea in blood, thermal status, and milk yield and composition of dairy cows under a high temperature humidity index environment.

2. Material and methods

The experiment was conducted between January and March (2016) at the Dairy Cattle Research Laboratory (Laboratório de Pesquisa em Bovinos de Leite) - Department of Animal Production and Animal Nutrition of the School of Veterinary Medicine and Animal Science, Pirassununga, Brazil. Experimental procedures were carried out under the approval of the Ethics Committee from the School of Veterinary Medicine and Animal Sciences, University São Paulo, São Paulo, Brazil (protocol #6134130415).

2.1. Animals and treatments

Thirty-six multiparous Holstein cows (of which twelve were ruminally cannulated) with 201 ± 63 days in milk, 599 ± 78 kg body weight, and 28.7 ± 3.92 kg/d milk yield (mean ± SD, at the start of experiment) were enrolled to this study. The experimental period lasted 6 weeks and consisted of one week for adaptation to the basal diet, and 5 weeks for data collection. During the entire experiment, cows were housed in a barn with individual concrete floor pens (17.5 m^2 of area) containing individual feed bunks, free access to water, and sand bedding. All cows received the same basal diet consisted of 50:50 corn silage to concentrate ratio, formulated according to National Research Council (NRC), 2001 recommendations (Table 1). During the first week of experiment, all animals received the same basal diet with no additives whereas the data of feed intake, milk yield and composition, and blood metabolites were used for covariate adjustments. After the first week, animals were blocked according to ruminal cannula presence, milk yield, days in milk, and body weight. Then, cows within each block (n = 12) were randomly assigned to one of the following treatments: basal diet (control; CON), no feed additives; functional oils (FO), basal diet added 500 mg/kg DM Essential® (Oligo basics, Cascavel, Brazil; a blend of CNSL and CO, with 200 g/kg of cardanol, 40 g/kg of cardol, and 90 g/kg ricinoleic acid as the bioactive components); and monensin (MON), basal diet added 22 mg/kg DM monensin (Rumensin, Elanco Animal Health, Greenfield, IN). Additives were added to the concentrate along with the mineral premix and diet was fed as a total mixed ration twice a day.

2.2. Temperature humidity index

Dry bulb temperature (Tdb, °C) and air relative humidity (RH, %) were measured during the collection period every 30 min using two data-loggers (Hobo® U12, Bourne, MA) placed at 2 m height in both sides of the barn. Temperature humidity index (THI) was calculated as follows (Mader et al., 2006):

$$THI = (0.8 \times Tdb) + \left[\left(\frac{RH}{100}\right) \times (Tdb - 14.6) + 46.4\right]$$

2.3. Feed intake and total tract apparent digestibility

Cows were fed twice daily (0800 and 1300 h) to maintain refusals between 5 and 10% (on as-fed basis) of feed provided on the previous day. Refusals were weighed daily to determine feed intake. Corn silage and ort samples were collected daily and composited by week. Ingredients in the concentrate were collected once a week. Fecal samples were collected directly from the rectum of cows during three consecutive days (9 h intervals) on weeks 3 and 5 of experiment. Fecal samples were frozen at −20 °C after each sampling. At the end of weeks 3 and 5, samples were thawed and composited (on a wet-basis) for each cow by week. Samples of feeds, orts, and feces were dried in a forced-air oven (72 h at 60 °C) and ground in a Wiley mill (MA340, Marconi, Piracicaba, Brazil) either through a 2-mm or 1-mm sieve.

Samples ground through 1-mm sieve were analyzed for DM (method 930.15), crude protein (CP; N × 6.25; Kjeldahl method 984.13), ether extract (method 920.39) and ash (method 942.05; AOAC, 2000) contents according to AOAC International (2000). Neutral detergent fiber (aNDF; with α-amylase without sodium sulfite addition), acid detergent fiber (ADF; Van Soest et al., 1991), and lignin (sulfuric acid method) were determined according to Goering and Van Soest (1970). Indigestible neutral detergent fiber

(iNDF) was used as internal marker to estimate daily DM fecal excretion. Samples of feed, orts, and feces (ground through 2-mm sieve) were placed in bags of non-woven fabric (5 × 5 cm and 100 g/m^2; America TNT, Foz do Iguaçú, Brazil; Casali et al., 2008) and incubated in the rumen of two Holstein cows for 288 h (Huhtanen et al., 1994). After the incubation period, samples were washed in running tap water and analyzed for NDF content as described earlier. Fecal excretion was estimated based on iNDF intake and iNDF fecal excretion, and total-tract digestibility was calculated based on nutrient intake and nutrient excretion.

2.4. Ruminal fermentation

On the last day of experiment, ruminal digesta was collected manually (cranial, dorsal, ventral caudo-dorsal, and caudo-ventral regions of the rumen) from the cannulated cows (n = 12) before (time 0), and 2, 4, 6, 8, 10, 12, 14, and 16 h after the morning feeding. Digesta was squeezed through four layers cheesecloth to extract ruminal fluid (250 ml). Ruminal fluid pH was immediately measured using a digital pH meter (MB-10®, Marte Científica, Santa Rita do Sapucaí, Brazil) and samples were frozen at −20 °C for further analyses of NH_3-N and VFA. Frozen ruminal fluid samples were thawed and centrifuged at 500*g* for 15 min. Supernatant aliquots (2 ml) were mixed with sulfuric acid (1 ml at 1 N) and analyzed for NH_3-N concentration by the colorimetric phenol-hypochlorite method (Broderick and Kang-Meznarich, 1980) and absorbances were measured using a microplate reader (Asys UVM 540, Biochorm, Cambridge, UK). Aliquots (1.6 ml) of ruminal fluid were mixed with formic acid (0.4 ml, p.a.) for VFA analysis according to Ferreira et al. (2018). VFA concentrations in ruminal fluid samples were determined using a gas chromatograph (Agilent 7890 A, Agilent Technologies, Santa Clara, CA) equipped with flame ionization detector (7683B, Agilent Technologies) and a fused-silica capillary column (J&W 19091 F-112, Agilent Technologies), 25 m length and 320 μm internal diameter, containing 0.20 μ*M* cyanopropyl polysiloxane. Identification of VFA peaks was performed using ChemStation software (Agilent Technologies).

2.5. Blood glucose and urea concentrations and thermal status parameters

Blood samples were collected from all cows by tail blood vessel puncture on weeks 1, 4, and 6 (on day 4 of each week), always 4 h after the morning feeding. Blood samples were collected in vacutainer tubes for serum and centrifuged (2000*g* for 15 min; FANEM® Excelsa 206 BL, São Paulo, Brazil) after clotting. Blood glucose and urea concentrations were analyzed using colorimetric enzymatic kits (K-082 and K-056, respectively; Bioclin®, Belo Horizonte, Brazil; Bergmeyer, 1985) and absorbances were measured in a spectrophotometer (SBA 200, CELM®, São Caetano do Sul, São Paulo, Brazil), as described by Ferreira de Jesus et al. (2016). Thermal status parameters were measured before the afternoon feeding (between 1200 and 1300 h) on weeks 4 and 6. Respiration rate was determined by counting flank movements during 1 min with a stopwatch. Heart rates were obtained with a stethoscope placed on the left chest wall in the region of the heart. Rectal temperatures were obtained using a clinical thermometer.

2.6. Milk production and composition

Cows were milked twice daily at 0600 and 1600 h, and milk yield was electronically recorded (Alpro®, DeLaval, Tumba, Sweden). Milk samples were collected automatically (proportionally to the milk yield and milk flow) during six consecutive milkings (three consecutive days) of every week. Milk samples were pooled per day, analyzed for milk components, and values averaged per week. Milk samples were analyzed for concentrations of fat, lactose, and true protein using an infrared milk analyzer (Lactoscan®, Entelbra, São Paulo, Brazil). Milk yield was corrected for 3.5% fat content according to Sklan et al. (1992).

2.7. Statistical analyses

Data were analyzed as repeated measures over time using the MIXED procedure of SAS (v ɯɷɷersion 9.3, SAS Institute and Inc, 2012), according to the following model:

$$Y_{ijk} = \mu + T_i + b_j + \omega_{ij} + B_1(X_{ij} - \bar{X})W_k + W \times T_{ki} + e_{ijk}$$

with: $b_j \approx N(0; \sigma_j^2)$, $\omega_{ij} \approx N(0; \sigma_\omega^2)$ and $e_{ijk} \approx MVN(0; R)$; where: Y_{ijk} = dependent variable; μ = overall mean; T_i = fixed effect of treatment (i = 1–3); b_j = random effect of block (j = 1–12); ω_{ij} = random error associated to experimental units (cow); B_1 is a fixed effect of regression coefficient; X_{ij} is the covariate measurement for cow; $\bar{X}$ is the overall mean of the covariate measurements; W_k = fixed effect of week (k = 1–5); $W \times T_{ki}$ is the interaction effect between week and treatment; e_{ijk} = residual error; N stands for Gaussian distribution; σ_j^2 = variance associate to block; σ_ω^2 = variance associated the random effect of cow; *MVN* stands for multivariate normal; and R is the variance-covariance matrix of residuals due to the repeated measurements. The following variance-covariance matrices were tested: CS, CSH, AR(1), ARH(1), TOEP, TOEPH, UN, FA(1) and ANTE(1). Matrix was chose by selecting the one with the lowest AICC value. Means were adjusted by the LSMEANS option and differences were protected LSD means test. Similar statistical analysis was performed for data related to nutrient digestibility and ruminal fermentation. For the nutrient digestibility data, the fixed effect of week accounted only for weeks 3 and 5 of experiment. For ruminal fermentation variables (pH, NH_3-N, and VFA), data were analyzed using the previous statistical model replacing the fixed effect of week with fixed effect of hour (0, 2, 4, 6, 8, 10, 12, 14, and 16 h). No covariate adjustments were performed for nutrient digestibility and ruminal fermentation data. Statistical differences were declared at $P \leq 0.05$ and tendencies considered when $0.05 < P \leq 0.10$.

3. Results

The barn temperature, RH, and THI values during the experiment were 25.0 ± 0.25 °C, 81.7 ± 1.17%, and 74.6 ± 0.28 (mean ± SE), respectively (Fig. 1). Cows fed MON had lower ($P \leq 0.021$) intake of DM and organic matter than cows in CON and FO groups (Table 2). Cows fed MON tended to decrease ($P = 0.075$) in CP intake when compared to CON. Treatments did not influence apparent total tract digestibility of nutrients. No interaction effect between treatment and time was observed for DM intake (Fig. 2). Treatments did not affect ruminal pH and NH_3-N concentration (Table 3). Feed additives (MON and FO) neither influenced ruminal VFA proportions nor total VFA concentration. No interaction effect between treatment and time was observed for ruminal fermentation variables.

Although no treatment effect was observed on blood glucose concentration, both MON and FO increased ($P = 0.006$) blood urea concentration (Table 4). No treatment effects were observed on heart rate, respiration rate, and rectal temperature. Feed additives had no effect on yields of milk, FCM, and solids, except for fat (Table 5). Cows fed FO had greater ($P = 0.021$) milk fat content compared to those fed MON, and FO tended to increase ($P \leq 0.066$) milk fat production in relation to MON. No interaction effect between treatment and time was observed on milk yield and composition, and feed efficiency.

4. Discussion

This study was designed to evaluate the impacts of FO on performance and thermal status of cows under heat stress. Our research group reported that FO can enhance the performance of cows not affected by heat stress; presumably due to an increase of propionate concentration in the rumen (Ferreira de Jesus et al., 2016). We expected that this positive effect on performance would be more evident in cows under heat stress due to FO antioxidant and gastroprotective properties. Indeed, cows in the present study experienced heat-stress where all daily values of THI were above 70 and the average THI during the entire experiment was 74.6. High producing cows are impacted by heat stress when THI exceeds 68 (Zimbelman et al., 2009) with negative effects on DM intake, nutrient digestibility, energy partitioning, and metabolism (Kargar et al., 2015). In this study, MON decreased DM intake of cows by 7.8%, an effect well documented in ruminants (Ipharraguerre and Clark, 2003). A meta-analysis from 53 trials demonstrated that MON reduces DM intake of cows by 2.3% (Duffield et al., 2008a). The reduction in DM intake promoted by MON was also reported in cows impacted by heat stress (THI values between 73 and 82; Baumgard et al., 2011). Although limited data is available, feeding FO to ruminants have not been reported to alter the DM intake of cows (Ferreira de Jesus et al., 2016; Zotti et al., 2017).

We expected that FO supplementation would promote a beneficial effect on nutrient digestibility under heat stress either due to changes in rumen microbial population (antimicrobial effect) or because of improvements in rumen/gut health status (antioxidant effect) of cows. For instance, authors have reported that plant extracts with antioxidant properties can reduce oxidative stress, promoting conditions for microbial growth and ruminal fermentation (Soltan et al., 2018, 2017). Under heat stress, blood diverts from the gut to periphery in an attempt to dissipate heat (Lambert et al., 2002), causing intestinal hypoxia. Intestinal epithelial cells

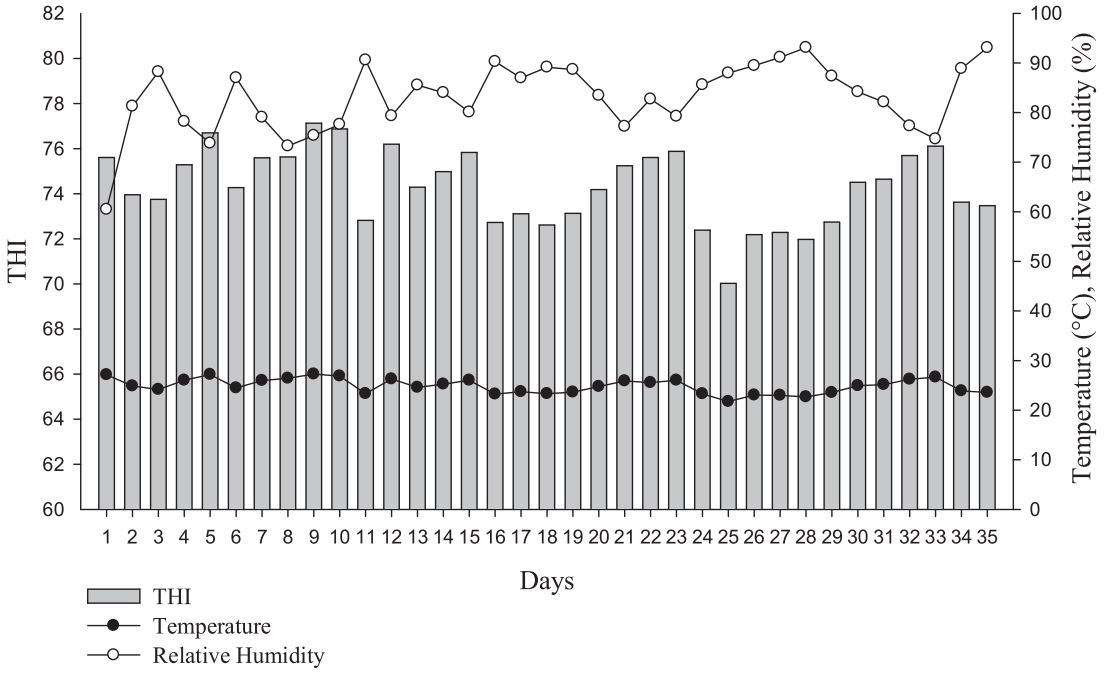

Fig. 1. Average daily temperature, relative humidity, and temperature humidity index (THI) throughout weeks 2–6 of experiment (treatment period).

Table 2
Effects of monensin and functional oils on nutrient intake and total tract digestibility in mid-lactation cows.

Item	Treatment[a]			SEM	*P*-value		
	CON	MON	FO		Treatment	Time	Treatment × time
Intake (kg/d)							
Dry matter	24.1[a]	22.2[b]	23.6[a]	0.286	0.021	0.482	0.419
Organic Matter	22.2[a]	20.4[b]	21.6[a]	0.262	0.020	0.027	0.411
NDF[b]	6.68	6.21	6.35	0.123	0.298	0.793	0.218
Crude Protein	4.10[c]	3.78[d]	3.94[cd]	0.070	0.075	< 0.001	0.815
Apparent total tract digestibility (g/kg)							
Dry matter	0.620	0.638	0.605	0.0085	0.315	0.012	0.361
Organic Matter	0.648	0.668	0.631	0.0078	0.187	0.011	0.272
Crude Protein	0.614	0.634	0.585	0.0104	0.180	0.002	0.532
NDF[b]	0.322	0.338	0.314	0.0123	0.699	< 0.001	0.255

a-bLSD means test with alpha 0.05.
c-dLSD means test with alpha 0.10.
[a] Treatments: control (CON), basal diet without additives; monensin (MON), 22 mg/kg DM Rumensin (Elanco Animal Health, Greenfield, IN); and functional oils (FO), 500 mg/kg DM Essential® (Oligobasics, Cascavel, Brazil).
[b] Neutral detergent fiber.

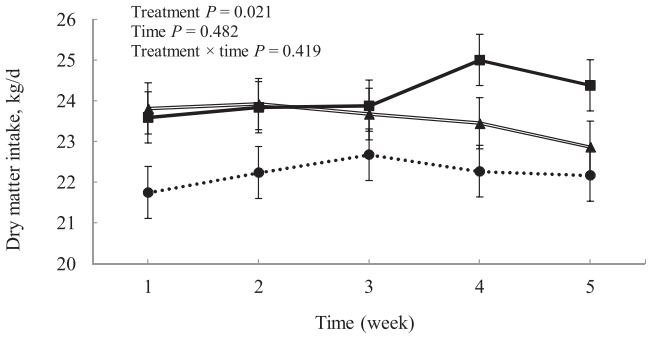

Fig. 2. Dry matter intake through the experimental period of cows fed control (CON; —■—), monensin (MON;···●··), and functional oil (FO;—▲—). Treatments: control (CON), basal diet without additives; monensin (MON), 22 mg/kg DM Rumensin (Elanco Animal Health, Greenfield, IN); and functional oils (FO), 500 mg/kg DM Essential® (Oligobasics, Cascavel, Brazil). Error bars are SE.

Table 3
Effects of monensin and functional oils on ruminal fermentation in mid-lactation cows.

Item	Treatment[a]			SEM	*P*-value		
	CON	MON	FO		Treatment	Time	Treatment × time
pH	5.94	6.02	5.94	0.038	0.396	< 0.001	0.461
NH_3-N (mg/dL)	11.8	13.7	16.3	1.14	0.197	< 0.001	0.299
VFA[b] (M/M)							
Acetate	0.545	0.536	0.558	0.005	0.110	0.022	0.999
Propionate	0.248	0.246	0.223	0.007	0.274	0.034	0.908
Butyrate	0.156	0.170	0.166	0.004	0.429	0.001	0.458
BCFA[c]	0.051	0.049	0.052	0.001	0.629	< 0.001	0.357
Total VFA (m*M*)	76.6	78.5	77.4	3.45	0.948	< 0.001	0.267
C2:C3 ratio[d]	2.23	2.19	2.52	0.077	0.176	0.002	0.872

[a] Treatments: control (CON), basal diet without additives; monensin (MON), 22 mg/kg DM Rumensin (Elanco Animal Health, Greenfield, IN); and functional oils (FO), 500 mg/kg DM Essential® (Oligobasics, Cascavel, Brazil).
[b] VFA: volatile fatty acids.
[c] BCFA: branched-chain fatty acids (isovalerate and isobutyrate).
[d] Acetate to propionate ratio.

are particularly sensitive to hypoxia, leading to ADP depletion and increased oxidative stress (Hall et al., 2001). The present study did not show evidence that FO improved total tract apparent digestibility of nutrients or altered rumen microbial population, since no FO effects were observed for ruminal fermentation variables. Indeed, feeding FO to dairy or beef cattle neither altered total tract apparent digestibility of nutrients (Valero et al., 2014; Branco et al., 2015; Ferreira de Jesus et al., 2016) or ruminal bacterial

Table 4
Effects of monensin and functional oils on serum metabolites, rectal temperature, heart and respiratory rates in mid-lactation cows.

Item	Treatment[a]			SEM	*P*-value		
	CON	MON	FO		Treatment	Time	Treatment × time
Metabolic profile							
Serum glucose (mg/dL)	70.6	71.2	68.9	1.94	0.879	0.598	0.717
Serum urea (mg/dL)	19.1[b]	22.1[a]	22.3[a]	0.43	0.006	0.812	0.044
Thermal status parameters							
Heart rate (min^{-1})	88.9	89.9	84.5	2.34	0.615	0.029	0.582
Respiration rate (min^{-1})	87.4	87.1	84.9	1.37	0.724	0.261	0.176
Rectal temperature (°C)	39.3	39.3	39.2	0.06	0.782	0.231	0.417

a-bLSD means test with alpha 0.05.
c-dLSD means test with alpha 0.10.
[a] Treatments: control (CON), basal diet without additives; monensin (MON), 22 mg/kg DM Rumensin (Elanco Animal Health, Greenfield, IN); and functional oils (FO), 500 mg/kg DM Essential® (Oligobasics, Cascavel, Brazil).

Table 5
Effects of monensin and functional oils on milk production and composition o mid-lactation cows.

Item	Treatment[a]			SEM	*P*-value		
	CON	MON	FO		Treatment	Time	Treatment × Time
Production (kg/d)							
Milk yield	29.2	29.5	29.1	0.252	0.786	0.002	0.828
3.5 % FCM[b]	28.6	28.2	29.6	0.346	0.269	0.002	0.736
Lactose	1.31	1.38	1.33	0.013	0.119	< 0.001	0.702
Fat	0.986[cd]	0.952[d]	1.042[c]	0.015	0.066	0.041	0.406
Protein	0.877	0.919	0.884	0.008	0.107	< 0.001	0.752
Milk composition (g/kg)							
Lactose	45.9	46.1	46.1	0.007	0.302	< 0.001	0.127
Fat	34.6[ab]	32.5[b]	36.5[a]	0.559	0.021	0.021	0.930
Protein	30.7	30.8	30.7	0.044	0.449	< 0.001	0.051
FCM:DMI[c]	1.19	1.26	1.22	0.064	0.407	0.016	0.366

[a−b]LSD means test with alpha 0.05.
[a] Treatments: control (CON), basal diet without additives; monensin (MON), 22 mg/kg DM Rumensin (Elanco Animal Health, Greenfield, IN); and functional oils (FO), 500 mg/kg DM Essential® (Oligobasics, Cascavel, Brazil).
[b] FCM: Fat-corrected milk.
[c] FCM:DMI: Fat-corrected milk to dry matter intake ratio.

populations (Zotti et al., 2017). Contrasting with the current study, authors have reported an increase in ruminal propionate molar proportion in both in vivo (Ferreira de Jesus et al., 2016) and in in vitro studies (Watanabe et al., 2010; Seradj et al., 2018) that added FO to diets/substrate. Authors have associated the latter effect with the antimicrobial effect of phenolic compounds such as anacardic acid isomers (Watanabe et al., 2010). In the current study, the lack of treatment effects on ruminal VFA profile could also be associated with the day of ruminal fluid sampling (last day of experiment) which does not account for interactions between treatment and time throughout the experiment.

Feed additives (MON and FO) increased blood urea concentration without affecting blood glucose levels. This result is likely associated with the numerical increase in ruminal NH_3-N concentration observed for MON and FO compared to CON (13.7, 16.3, and 11.8 mg/dL, respectively). The greater numerical values of ruminal NH_3-N concentration for cows fed MON when compared to CON was not expected since MON is known to inhibit ruminal bacteria that deaminate proteins (Schelling, 1984). However, a meta-analysis from 23 trials speculated that MON increases blood urea concentration due to an improved ability to liver synthesize urea in lactating cows (Duffield et al., 2008b). In terms of FO supplementation, authors have reported a decrease in blood urea concentration (Gandra et al., 2014; Ferreira de Jesus et al., 2016). Zotti et al. (2017) reported a decrease in population of ciliated protozoa when feeding FO to beef cattle. Decreasing the population of ciliated protozoa reduces ruminal NH_3-N concentration (Firkin et al., 2007). The reasons for the greater ruminal NH_3-N concentration in cows fed FO compared to those in CON are not clear.

Panting/high respiration rates can contribute to rumen acidosis due to an imbalance of blood pH buffering system. Hyperventilation reduces blood CO_2 concentration while kidneys increase the excretion of HCO_3^- to maintain an optimum HCO_3^- to CO_2 ratio (20:1). Therefore, less HCO_3^- is available for saliva and consequently to buffer rumen contents (Kadzere et al., 2002; Conte et al., 2018). Although animals fed FO exhibited numerically lower values of heart and respiration rates, no significant differences were observed in these variables. Agreeing with the current study, Boyd et al. (2012) reported no effects of plant extracts on thermal status of dairy cows under heat stress (THI 78.8). Similarly, Havlin and Robinson (2015) reported no effects of a citrus extract on the thermal status of heat-stressed dairy cows. The current study provides no clear evidence that FO can alter respiration rates and rumen pH of cows.

Treatments neither affected milk yield nor milk protein content. However, FO increased milk fat concentration and tended to increase milk fat production in comparison with MON. The numerical changes observed in VFA profile are likely related to the increase in milk fat production. MON modulated VFA profile towards a greater propionate and butyrate concentration, whereas FO apparently modulated the VFA profile towards a greater concentration of acetate instead of propionate. Acetate is the main substrate for de novo milk fat synthesis and plays a critical role on stimulating mammary lipogenesis under conditions of normal milk fat content (Urrutia and Harvatine, 2017). The numerical increase in ruminal acetate proportion which may reflect an increase in ruminal fiber digestibility (not evaluated in the current experiment), especially because treatments had no effect on BW change and body condition score of cows (data not shown). Evidence suggests that FO can alter fat synthesis in ruminants. For instance, authors have reported an increase in back fat thickness and carcass grade of beef cattle fed FO (Purevjav et al., 2013) and a study reported an increase in milk fat concentration when feeding CO to dairy cows (Gandra et al., 2014).

5. Conclusion

Functional oils increased milk fat production, and can be used in place of monensin without affecting feed intake, digestion, ruminal fermentation, and thermal status of dairy cows under a high THI environment. On the other hand, MON decreased feed intake and had no effect on animal performance.

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