Feeding oregano oil and its main component carvacrol does not affect ruminal fermentation, nutrient utilization, methane emissions, milk production, or milk fatty acid composition of dairy cows

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ABSTRACT

Because of their antimicrobial properties, essential oils and their components have been suggested as alternatives to other antimicrobials (e.g., monensin) that are commonly fed to ruminants to improve nutrient utilization and enhance feed efficiency and milk performance. In this study, we evaluated the potential of oregano oil and its main component (carvacrol) as rumen modifiers. For this purpose, 8 ruminally cannulated lactating dairy cows (92 ± 11 d in milk, 36.5 ± 7.6 kg of milk yield, and 703 ± 74 kg of body weight) were used in a double 4×4 Latin square (28-d periods). Cows were fed 1 of the 4 following treatments: (1) control (CTL, no additive); (2) monensin [MON, 24 mg/kg of dry matter (DM)]; (3) oregano oil (ORE, 50 mg/kg of DM); and (4) carvacrol (CAR, 50 mg/kg of DM). Cows were fed (ad libitum intake) a total mixed ration consisting of 60% forages (corn silage and alfalfa silage) and 40% concentrates, on a DM basis. Feeding ORE and CAR had no effect on nutrient total-tract apparent digestibility, N utilization, rumen fermentation (i.e., pH, ammonia, volatile fatty acids), protozoa counts, or milk performance. Feeding MON increased the molar proportion of propionate and tended to increase total-tract apparent digestibility of crude protein. None of the feed additives evaluated affected enteric methane production (491 g/d, 21.1 g/kg of DM intake, 6.14% of gross energy intake on average). Milk fatty acid composition was not changed by ORE or CAR, but MON increased the proportion of *trans*-10 18:1, an intermediate of ruminal biohydrogenation. Thus, when included at 50 mg/kg of dietary dry matter, neither oregano oil nor carvacrol favorably altered rumen fermentation, improved nutrient utilization or milk performance, or mitigated enteric methane emissions in dairy cows.

Key words: essential oils, nutrient utilization, milk production, milk fatty acid, methane production

INTRODUCTION

Because of increasing public pressure to decrease the use of antimicrobials in livestock production and the regulations that ban the use of these substances in Europe (European Union, 2003), scientists and the livestock feed industry have been actively working to find alternatives to antimicrobials (Benchaar et al., 2008, 2009). Among these alternatives, essential oils (EO) and their compounds have attracted much attention because of their antimicrobial properties that may modulate rumen fermentation (Benchaar and Greathead, 2011). Essential oils (e.g., oregano oil) with high concentrations of phenolic compounds (e.g., carvacrol) have been evaluated, mainly in vitro, as rumen fermentation modifiers (see reviews by Calsamiglia et al., 2007 and Cobellis et al., 2016). Several short-term in vitro studies have shown that oregano oil and its main component carvacrol affect N metabolism via the reduction of protein degradation and ammonia production (Benchaar et al., 2008). Oregano oil and carvacrol have also been investigated extensively for their potential to inhibit ruminal methanogenesis in vitro (Benchaar and Greathead, 2011; Cobellis et al., 2016). For instance, using in vitro batch cultures of ruminal fluid, Macheboeuf et al. (2008) observed that oregano oil decreased methane production (up to a 98% decrease). In the same study, the authors reported lower antimethanogenic activity of carvacrol, suggesting that other components present in lower concentrations in oregano oil may have acted antagonistically with carvacrol, thereby attenuating the antimethanogenic properties of oregano oil. However, because of the limitations of in vitro techniques (i.e., short-term incubation, buffered medium, inability to replicate the diversity and viability of the microbial population of the rumen), data should be interpreted with caution. Furthermore, the high concentrations used in vitro are impractical for in

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vivo conditions; thus, the effects observed in vitro may not be reliable for in vivo applications (Beauchemin et al., 2009; Benchaa and Greathead, 2011).

Few in vivo studies have investigated the use of oregano oil in dairy cows and, to the best of our knowledge, no studies have been published on the potential use of carvacrol as a feed additive in dairy cow diets. In a Danish study, Lejonklev et al. (2016) observed no changes in DMI, milk production, or enteric CH₄ emissions in dairy cows (n = 3) fed high concentrations of oregano oil for 1 d (0.2 and 1.0 g/kg of DM, corresponding to intakes of 4.3 and 22.4 g/d, respectively). Tekippe et al. (2011) and Hristov et al. (2013) assessed the effects of oregano leaves on rumen fermentation, nutrient digestibility, N excretion, CH₄ production, and milk performance of dairy cows. In Tekippe et al. (2011), ruminal pulse dosing of 500 g/d of oregano leaves had no effect on DMI, milk yield, VFA (total and individual) concentrations, protozoa number, nutrient digestibility, or N excretion. Enteric CH₄ production measured within 8 h after feeding using the SF₆ tracer technique and sampling the rumen headspace decreased with oregano oil supplementation. In a subsequent study by the same group, supplementation of oregano leaves (250, 500, and 750 g/d) decreased DMI but had no effect on nutrient digestibility, rumen fermentation characteristics, protozoa, N excretion, or milk fatty acid (FA) profile (Hristov et al., 2013). Using the same methodology as Tekippe et al. (2011), Hristov et al. (2013) reported a decrease in enteric CH₄ production in cows fed increasing amounts of oregano leaves (250, 500, and 750 g/d). In both studies, inhibition of CH₄ production was not associated with a shift in rumen fermentation toward propionogenesis and had no effect on protozoa or *Methanobrevibacter*, the predominant *Archaea* genus in the rumen. All of these factors are known to influence ruminal methanogenesis (Beauchemin et al., 2009). Furthermore, given that CH₄ was measured only within 8 h after feeding, it is not certain that the inhibitory effect of oregano leaves would have persisted during the remaining 16 h of the feeding cycle (Tekippe et al., 2011; Hristov et al., 2013). More recently, Kolling et al. (2018) fed oregano extract (10 g/d) to dairy cows and reported no change in DMI, nutrient digestibility, milk yield, or milk composition. During the gas measurement period in the respiration chambers, Kolling et al. (2018) observed a decline in enteric CH₄ production (g/kg of DMI) associated with a tendency for an increase in DMI.

The effects of oregano leaves or extract on enteric CH₄ observed by Tekippe et al. (2011), Hristov et al. (2013), and Kolling et al. (2018) cannot be attributed solely to the EO fraction or its main component car-

vacrol, or both (up to 90.8% of the oil; Tekippe et al., 2011) contained in the oregano supplement. In fact, compounds other than EO may have contributed to the inhibitory effect of oregano leaves or extract. Oregano may also contain other substances such as lipids, FA, phenolic, quinones, and flavonoids (Kerala, 2001; Skoula and Harborne, 2002; Skoula et al., 2008). Many of these substances, in particular flavonoids, have been shown to exhibit antimicrobial activities (Baričević and Bartol, 2002; Cushnie and Lamb, 2005; Skoula et al., 2008) and thus, may also inhibit rumen methanogens. In contrast, in the studies by Tekippe et al. (2011) and Hristov et al. (2013), the concentrations of EO and carvacrol provided by oregano leaves ranged from 4 to 12 g/d (140 to 445 mg/kg of DMI) and 3 to 9 g/d of carvacrol (110 to 350 mg/kg of DMI), respectively. Such doses of EO are higher than the levels recommended by feed additive suppliers and the doses reported in the literature (Benchaa et al., 2009; Cobellis et al., 2016) and are not cost effective if oregano oil or carvacrol is used as a feed additive in dairy cow diets.

Based on these considerations, the aim of this study was to evaluate the potential of using oregano oil and its main constituent carvacrol as feed additives in dairy cow diets to modulate rumen fermentation, improve feed efficiency, reduce N excretion, and mitigate enteric CH₄ production. Accordingly, the specific goals were to determine the effects of oregano oil and carvacrol on feed intake, ruminal fermentation, nutrient total-tract apparent digestibility, N excretion, CH₄ production, milk production, and milk FA composition.

MATERIALS AND METHODS

Cows, Experimental Design, and Diets

Eight multiparous cows fitted with rumen cannulas (10 cm i.d.; Bar Diamond Inc., Parma, ID) were used in this study. The experimental design was a replicated 4 × 4 Latin square design balanced for residual effects (Cochran and Cox, 1957). At the start of the trial, cows averaged (mean ± SD) 92 ± 11 DIM, 36.5 ± 7.6 kg of milk yield, and 703 ± 74 kg of BW. Cows were kept in individual tie stalls and had free access to water for the duration of the experiment. They were fed the same basal diet as a TMR (Table 1). Experimental treatments were (1) TMR with no additive (CTL); (2) TMR supplemented with monensin (MON; 24 mg/kg); (3) TMR supplemented with oregano oil (ORE; 50 mg/kg); and (4) TMR supplemented with carvacrol (CAR; 50 mg/kg).

Oregano oil (containing 70% carvacrol) and CAR (98% purity) were from Phodé S.A. (Albi, France); mo-

Table 1. Ingredient and chemical composition of the TMR

Item (% of DM, unless otherwise noted)	Value
Ingredient	
Alfalfa silage	31.4
Corn silage	28.7
Corn grain, rolled	21.7
Soybean meal, 48% solvent-extracted	7.64
Beet pulp, dehydrated	5.61
Top supplement ¹	2.63
Mineral and vitamin supplement ²	2.04
Calcium carbonate	0.25
Chemical composition	
OM	92.6
CP	17.9
NDF	31.3
ADF	20.8
Starch	20.0
Ether extract	2.66
Gross energy (Mcal/kg of DM)	4.43
NE _L (Mcal/kg of DM) ³	1.60
Fatty acid (g/kg of DM)	
14:0	0.09
16:0	5.26
cis-9 16:1	0.06
18:0	0.83
cis-9 18:1	5.49
cis-11 18:1	0.23
cis-9,cis-12 18:2	12.2
cis-9,cis-12,cis-15 18:3	3.76
20:0	0.20
Total	28.1

¹Contained 20.8% corn gluten meal, 28.3% soybean meal Trituro (SoyaExcel, Saint-Charles-Sur-Richelieu, QC, Canada), 16.7% canola meal, 34.2% dried corn distillers grains.

²Contained 12.5% Ca, 6.80% P, 6.81% Mg, 2.49% S, 7.72% Na, 1.97% K, 2,877 mg/kg Fe, 3,777 mg/kg Zn, 620 mg/kg Cu, 2,520 mg/kg Mn, 96 mg/kg I, 83 mg/kg Co, 27.8 mg/kg Se, 628,000 IU/kg of vitamin A, 81,000 IU/kg of vitamin D, and 3,739 IU/kg of vitamin E.

³Calculated according to NRC (2001).

nensin (Rumensin 80) was from Elanco Animal Health (Guelph, ON, Canada). Each feed additive was pre-mixed with a portion of the concentrate and included in the TMR to obtain the targeted concentration. The diets were offered ad libitum (5% refusals on an as-fed basis). Adaptation to experimental treatments was from d 1 to 14, ruminal fluid sampling occurred on d 14 and d 28, CH₄ production monitoring from d 19 to 25, and sampling of milk, feces, and urine from d 20 to 26. The Institutional Animal Care Committee of the Sherbrooke Research and Development Centre (Sherbrooke, QC, Canada) approved the animal procedures and all cows were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009).

Intake, Total-Tract Apparent Digestibility, and N Excretion

Cows were fed daily at 0900 and 1930 h. Daily feed intake was determined by weighing the amounts of feed

offered and orts. Feed samples (i.e., TMR, ingredients, orts) were collected daily and kept at -20°C . They were later thawed, composited by cow within period, freeze-dried, and ground through a 1-mm screen (standard model 4; Arthur H. Thomas, Philadelphia, PA) for the analysis of DM, OM, total N, NDF, ADF, starch, ether extract, gross energy (GE), and FA.

Feces and urine were collected separately using harnesses and tubes, weighed, and sampled proportionally (2%, on a fresh matter basis), as described in Benchaar et al. (2013). The daily samples were stored at -20°C and later thawed and composited by cow within period. Fecal samples were freeze-dried and ground through a 1-mm screen for the analysis of DM, OM, total N, NDF, starch, and GE concentrations. Urine samples were frozen at -20°C until analyzed for total N.

Ruminal Fermentation Characteristics and Protozoa Enumeration

Ruminal pH, VFA, and ammonia were measured in ruminal fluid collected before (0 h) and at 1, 2, 4, 6, and 8 h after the a.m. feeding. For this purpose, ruminal fluid (~250 mL) was collected from the anterior dorsal, anterior ventral, medium ventral, posterior dorsal, and posterior ventral locations within the rumen using a 50-mL syringe screwed to a stainless tube ending with a probe covered by a fine metal mesh (RT Rumen Fluid Collection Tube, Bar Diamond Inc.). Ruminal pH was measured immediately after sampling (Accumet pH meter; Fisher Scientific, Montreal, QC, Canada). Samples (15 mL) were stored at -20°C for the determination of VFA. Ammonia was measured in samples conserved with 50% sulfuric acid and stored at -20°C until analysis.

Protozoa enumeration was performed as described in Benchaar et al. (2013). Ruminal content (~1 L) was collected 4 h after the a.m. feeding and squeezed through 4 layers of cheesecloth. Samples (5 mL) of the filtered ruminal fluid were preserved with methyl green formalin-saline solution (5 mL) for later enumeration of protozoa (Ogimoto and Imai, 1981) using a counting chamber (Neubauer Improved Bright-Line counting cell, 0.1-mm depth; Hausser Scientific, Horsham, PA).

Milk Production and Milk Composition

Cows were milked daily at 0700 and 1900 h, and milk production was measured and recorded at each milking. Milk samples were taken at each milking, preserved with 2-bromo-2-nitropropan-1,3-diol, and shipped to Valacta (Ste-Anne-de-Bellevue, QC, Canada) for analysis of milk components (i.e., fat, protein, lactose)

and MUN. Samples pooled from 4 consecutive milkings proportionally to milk yield were frozen at -80°C for the determination of milk FA composition.

Enteric Methane Production

Enteric CH_4 production was measured using the sulfur hexafluoride (SF_6) tracer technique (Johnson et al., 1994) as described in McGinn et al. (2006). Briefly, at the start of the trial, a calibrated permeation tube containing SF_6 (4.479 ± 0.282 mg/d) was placed into the rumen of each cow and remained throughout the duration of the experiment. Breath samples of each cow were collected daily (d 19 to 25) in an evacuated canister (~ 2 L) through a tube placed near the nostrils of the cow. After 24 h, samples ($n = 3$; 20 mL/sample) were collected from the canister and kept in evacuated Exetainers (Labco Ltd., Buckinghamshire, UK) for the analysis of CH_4 and SF_6 . The canisters were changed every day before the a.m. feeding. Daily background concentrations of CH_4 and SF_6 in the barn were determined by placing canisters in different locations. Production of CH_4 of each cow was calculated as described in McGinn et al. (2006).

Chemical Analyses

Dry matter was measured at 100°C using a vacuum oven overnight (AOAC International, 2005; method 934.01), OM by incineration at 550°C overnight in a muffle furnace (AOAC International, 2005; method 942.05), and total N according to the macro-Kjeldahl procedure (AOAC, 2005; method 954.01). The NDF was determined according to Van Soest et al. (1991) with the inclusion of sodium sulfite and heat stable α -amylase. The ADF was determined according to AOAC International (2005; method 973.18). The NDF and ADF procedures were adapted for use in an Ankom200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Starch was determined colorimetrically according to the procedure described in AOAC International (2005; method 996.11). Ether extract was determined according to AOAC International (2005; method 920.39) using the Soxtec 2050 extraction system (Foss, Höganäs, Sweden). Gross energy was determined using an adiabatic bomb calorimeter (model 6200, Parr Instrument Company, Moline, IL). Total N in urine was determined by micro-Kjeldahl analysis (AOAC International, 2005; method 960.52). Volatile fatty acids and ammonia were determined by gas chromatography (Hewlett-Packard Labs, Palo Alto, CA) and according to the method of Weatherburn (1967), respectively, as described in Benchaar (2016). Milk concentrations of true protein, fat, lactose, and urea N were analyzed by

infrared spectroscopy (MilkoScan FT 6000; Foss Electric, Hillerød, Denmark).

For the analysis of FA in milk, FA were transmethyated and FAME were analyzed by GC (HP 5890A Series II, Hewlett Packard, Palo Alto, CA) as previously described in Chouinard et al. (1997) and Farnworth et al. (2007). Dietary FA were analyzed according to the technique of Sukhija and Palmquist (1988).

Methane and SF_6 were determined by chromatography as described in detail in Benchaar (2016). The GC (model CP-3800, Varian Inc., St-Laurent, QC, Canada) was equipped with a flame-ionization detector fitted with a column silica plot FS ($30 \text{ m} \times 0.32 \text{ mm}$) for CH_4 analysis, and with an electron capture detector fitted with a Hayesep D stainless steel column ($2 \text{ m} \times 2 \text{ mm}$; 80–100 mesh) for SF_6 analysis. Both columns were from Varian Inc. (Lake Forest, CA).

Statistical Analyses

All data were analyzed as replicated 4×4 Latin square design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Data of DMI, milk production, milk composition, digestibility, and CH_4 production were averaged across days and analyzed using the following model:

$$Y_{ijk} = \mu + A_i + P_j + T_k + e_{ijk},$$

where Y_{ijk} = dependent variable; μ = overall mean; A_i = random effect of cow ($i = 1$ to 8); P_j = fixed effect of period ($j = 1, 2, 3, 4$); T_k = fixed effect of treatment (CTL, MON, ORE, CAR); and e_{ijk} = residual error.

Data collected at different time intervals (i.e., pH, VFA, ammonia) were analyzed as repeated measures the using the following model:

$$Y_{ijklm} = \mu + A_i + P_j + T_k + H_l + T \times H_{kl} + e_{ijkl},$$

where H_l = fixed effect of sampling time (0, 1, 2, 4, 6, 8 h), $T \times H_{kl}$ is the fixed effect of sampling time $\times$ treatment interaction, and other terms are as defined above.

Statistical differences were declared significant at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$ using the Tukey correction test for multiple comparisons among treatments.

RESULTS

DMI, Total-Tract Apparent Digestibility, and N Excretion

Data of DMI, nutrient digestibility and N excretion are shown in Table 2. Diet supplementation with MON,

Table 2. Dry matter intake, nutrient total-tract apparent digestibility, and N excretion in lactating cows fed a TMR without supplementation (control, CTL), or supplemented with monensin (MON), oregano oil (ORE), or carvacrol (CAR)

Item	Treatment				SEM	P-value
	CTL	MON	ORE	CAR		
DMI (kg/d)	23.8	23.3	24.2	23.7	0.45	0.55
Digestibility (%)						
DM	68.1	68.8	68.4	68.3	0.43	0.73
OM	69.6	70.3	69.8	69.7	0.38	0.50
CP	66.6†	68.3†	67.2	66.7	0.50	0.10
NDF	50.3	50.6	49.6	51.8	0.98	0.46
Gross energy	66.5	67.3	66.7	66.6	0.39	0.53
Excretion (g/d)						
Feces	229	212	229	225	5.4	0.12
Urine	215	214	215	221	5.4	0.76
Total	444	426	444	446	9.7	0.42

† $P = 0.10$: MON vs. CTL.

ORE, and CAR had no effect on DMI (23.8 kg/d), total-tract apparent digestibility of DM (68.4%), OM (69.9%), NDF (50.6%), or GE (66.8%). Total-tract apparent digestibility of CP tended ($P = 0.10$) to be higher for cows fed MON than for cows fed CTL (68.3 vs. 66.6%). The inclusion of the feed additives in the diet had no effect on N intake (683 g/d) or on excretion of N in feces (224 g/d) or urine (216 g/d). Similarly, total N excretion (i.e., sum of fecal and urinary N) was not changed by experimental treatment and averaged 440 g/d.

Ruminal Fermentation Characteristics and Protozoa

The effects of experimental treatments on ruminal fermentation characteristics and protozoa are shown

in Table 3. Neither ruminal pH (6.20, on average) nor total VFA concentration (141.9 mM, on average) was changed by feeding cows MON, ORE, or CAR. Molar proportions of acetate (61.5 mol/100 mol), butyrate (12.5 mol/100 mol), branched-chain VFA (2.58 mol/100 mol), and caproate (0.60 mol/100 mol) were not affected by the inclusion of feed additives in the diet. The molar proportion of propionate was higher for cows fed MON than for cows fed CTL (22.6 vs. 20.8 mol/100 mol). Consequently, the acetate-to-propionate ratio was lower ($P < 0.05$) for cows fed MON than for cows fed CTL (2.71 vs. 3.03). The molar proportion of propionate tended to be lower for cows fed ORE ($P = 0.10$) and CAR ($P = 0.09$) than for cows fed MON (21.1 and 21.0 vs. 22.6%). Ammonia concentration (7.71 mM, on average) and protozoa count (7.61 log₁₀/mL, on average) were similar among cows.

Table 3. Ruminal fermentation characteristics and protozoa counts of lactating cows fed a TMR without supplementation (control, CTL), or supplemented with monensin (MON), oregano oil (ORE), or carvacrol (CAR)

Item	Treatment				SEM	P-value
	CTL	MON	ORE	CAR		
pH	6.22	6.19	6.19	6.19	0.027	0.88
Total VFA (mM)	140.2	141.1	143.9	142.4	2.59	0.76
VFA (mol/100 mol)						
Acetate	62.1	60.5	61.7	61.7	0.54	0.25
Propionate	20.8 ^b	22.6 ^{a†}	21.1 ^{ab†}	21.0 ^{ab†}	0.43	0.03
Butyrate	12.5	12.2	12.5	12.7	0.30	0.75
Branched-chain VFA ¹	2.57	2.69	2.49	2.58	0.062	0.19
Valerate	1.49	1.46	1.53	1.47	0.039	0.59
Caproate	0.64	0.54	0.65	0.59	0.034	0.11
Acetate:propionate	3.03 ^a	2.71 ^b	2.95 ^{ab}	2.98 ^{ab}	0.081	0.05
Ammonia (mM)	8.17	7.30	7.60	7.78	0.409	0.52
Protozoa (log ₁₀ /mL)	8.44	6.49	8.09	7.42	0.779	0.33

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).¹Sum of isobutyrate and isovalerate.† $P = 0.10$: MON vs. ORE; $P = 0.09$: MON vs. CAR.

Enteric CH₄ Production

Data on the effect of the feed additives on enteric CH₄ production are presented in Table 4. Compared with CTL, diet supplementation with MON, ORE, or CAR did not affect enteric CH₄ production expressed in grams/day (491 g/d, on average) or on a DMI basis (21.1 g/d, on average). When expressed as a proportion of GE intake, CH₄ energy losses were similar for cows fed CTL, MON, ORE, and CAR (6.14% of GE intake, on average).

Milk Production and Milk Composition

Milk performance data are shown in Table 5. Milk production was not altered by the inclusion of MON, ORE, or CAR in the diet (34.2 kg/d, on average). Likewise, milk concentrations of fat (3.75%), lactose (4.61%), and MUN (10.5 mg/L) were similar among cows. For milk yield components, protein yield was lower ($P = 0.05$) for cows fed CAR compared with cows fed ORE (1.08 vs. 1.13 kg/d). None of the feed additives affected feed efficiency, which averaged 1.43 kg of milk/kg of DMI.

Milk Fatty Composition

Data on the effects on milk fatty composition (g/100 g) are shown in Table 6. Concentrations of 6:0 and 8:0 tended ($P = 0.08$ and 0.07 , respectively) to be lower in milk fat of cows fed MON than in those fed CTL (1.864 vs. 1.963 and 1.048 vs. 1.130 g/100 g, respectively). In contrast, the concentration of 17:0 was higher ($P < 0.05$) in milk fat of cows fed MON than in cows fed CTL (0.515 vs. 0.477 g/100 g). Concentrations of *trans*-9 18:1 and *trans*-10 18:1 were higher ($P < 0.01$) for cows fed MON (0.179 and 0.322 g/100 g, respectively) compared with cows fed CTL, ORE, or CAR (0.167 and 0.263 g/100 g for *trans*-9 18:1 and *trans*-10 18:1, on average, respectively). The concentration of

trans-11 18:1 was lower for cows fed MON than for cows fed ORE (0.707 vs. 0.793 g/100 g of fat; $P < 0.05$). The concentration of this FA tended ($P = 0.10$) to decrease when cows were fed CAR compared with ORE (0.718 vs. 0.793 g/100 g). The concentration of *trans*-12 18:1 was higher ($P < 0.05$) for cows fed MON than for cows fed CTL or CAR (0.397 vs. 0.359 and 0.347 g/100 g). Cows fed MON tended ($P = 0.11$) to have a higher concentration of *trans*-12 18:1 than cows fed ORE (0.397 vs. 0.366 g/100 g). Cows fed MON had higher ($P < 0.01$) concentrations of *trans*-15 18:1 and *trans*-16 18:1 (0.409 and 0.336 g/100 g, respectively) than cows fed CTL, ORE, and CAR (0.363 and 0.290 g/100 g for *trans*-15 18:1 and *trans*-16 18:1, on average, respectively). The concentration of *cis*-9,*trans*-11 18:2 decreased ($P < 0.01$) when cows were fed CAR compared with ORE (0.304 vs. 0.354 g/100 g). Cows fed MON had a higher concentration of *trans*-8,*cis*-13 18:2 compared with cows fed CTL, ORE, and CAR (0.081 vs. 0.66 g/100 g). Cows fed MON had higher ($P < 0.05$) concentrations of *trans*-9,*cis*-13 + *trans*-8,*cis*-12 18:2 compared with cows fed ORE or CAR (0.286 vs. 0.256 and 0.243 g/100 g, respectively). The concentration of *trans*-9,*cis*-13 + *trans*-8,*cis*-12 18:2 tended ($P = 0.08$) to increase in milk fat of cows fed MON versus those fed CTL (0.286 vs. 0.258 g/100 g). The ratio of *trans*-11 18:1 to *trans*-10 18:1 was lower ($P < 0.05$) for cows fed MON than for cows fed CTL, ORE, and CAR (2.241 vs. 2.875, respectively).

DISCUSSION

This study was undertaken to examine the potential of using oregano oil and its main component carvacrol as feed additives in dairy cow nutrition. Little information is available on the use of oregano oil in dairy cow diets and, to our knowledge, no studies have been published on the effects of pure carvacrol on ruminal fermentation, N excretion, enteric CH₄ production, and milk performance of dairy cows.

Table 4. Enteric CH₄ production in lactating cows fed a TMR without supplementation (control, CTL), or supplemented with monensin (MON), oregano oil (ORE), or carvacrol (CAR)

Item	Treatment				SEM	P-value
	CTL	MON	ORE	CAR		
DMI ¹ (kg/d)	23.4	22.6	23.8	23.2	0.46	0.23
CH ₄ g/d	488	462	511	502	16.6	0.14
g/kg of DMI	20.9	20.3	21.5	21.6	0.49	0.17
% of GE intake	6.02	5.89	6.33	6.34	0.153	0.08

¹Measured during enteric methane determination period (i.e., 7 d, each period).

Table 5. Milk production and milk composition of lactating cows fed a TMR without supplementation (control, CTL) or supplemented with monensin (MON), oregano oil (ORE), or carvacrol (CAR)

Item	Treatment				SEM	<i>P</i> -value
	CTL	MON	ORE	CAR		
Production (kg/d)	34.3	34.2	34.6	33.6	0.46	0.53
Composition (%)						
Fat	3.71	3.76	3.79	3.73	0.083	0.92
Protein	3.30	3.27	3.30	3.23	0.024	0.13
Lactose	4.62	4.60	4.62	4.62	0.020	0.84
Yield (kg/d)						
Fat	1.27	1.29	1.32	1.27	0.023	0.41
Protein	1.12 ^{ab}	1.11 ^{ab}	1.13 ^a	1.08 ^b	0.014	0.06
Lactose	1.59	1.58	1.61	1.56	0.024	0.59
MUN (mg/dL)	10.6	10.7	10.4	10.4	0.36	0.94
Feed efficiency (milk/DMI)	1.44	1.46	1.41	1.42	0.021	0.43

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

Effect on DMI, Total-Tract Apparent Digestibility, and N Excretion

Diet supplementation with ORE did not affect DMI or total-tract apparent digestibility of nutrients. Kolling et al. (2018) observed no change in DMI (measured outside the chambers) or digestibility of DM, fiber, CP, and GE in cows fed oregano extract (10 g/d; 0.056% of dietary DM; 5% EO, 80% carvacrol). When feeding dairy cows large amounts of dried oregano leaves (250 to 750 g/d), Hristov et al. (2013) observed a linear decrease in DMI. In Hristov et al. (2013), the concentration of EO in oregano leaves was 1.58% of DM, which means that cows were supplemented daily with 4 to 12 g/d of EO, corresponding to 140 to 445 mg/kg of dietary DM. This level of supplementation is 3 to 9 times higher than that used in the current study (50 mg/kg of DMI), which may explain the discrepancy between the 2 studies. Nitrogen excretion (i.e., urinary N, fecal N, and total) was unaffected by ORE supplementation, which is in accordance with findings by Hristov et al. (2013) in lactating cows supplemented with oregano leaves (250 to 750 g/d). In the present study, adding CAR to the diet had no effect on DMI and total-tract apparent digestibility of nutrients and N excretion. Information on feeding CAR to dairy cows is available. In one study with lambs, Chaves et al. (2008) observed no change in DMI when the animals were supplemented with 200 mg/kg of dietary DM of carvacrol.

The ionophore monensin has been extensively investigated in dairy cows. In the present study, feeding MON (24 mg/kg of DM) did not affect DMI, which agrees with the results of other studies with dairy cows fed MON at a feeding rate similar to that used in the current study (Grainger et al., 2010; Benchaar, 2016). Total-tract apparent digestibility of DM, OM, NDF,

and GE was unaffected by MON supplementation. Supplementing the diet with MON tended ($P = 0.10$) to increase CP digestibility, which is in agreement with previous studies with dairy cattle fed MON (Plaizier et al., 2000; Benchaar et al., 2006). In contrast, Plaizier et al. (2000) and Benchaar (2016) reported no effect of MON on CP digestibility in dairy cows. Differences between studies in CP digestibility response to MON may be due to diet composition (i.e., forage:concentrate ratio) or the dose of MON used (Benchaar et al., 2006; Benchaar, 2016).

Effect on Rumen Fermentation and Protozoa

Ruminal pH and VFA (total and individual) proportions were unaffected by feeding cows ORE and CAR. Feeding oregano leaves (500 g/d; Tekippe et al., 2011) or extract (10 g/d; Kolling et al., 2018) did not change ruminal pH and total VFA concentration. In our study and these previous studies, the lack of effect on ruminal pH and total VFA concentration was consistent with the absence of effects of the additives on DMI, rumen digestion, and total-tract apparent digestibility of nutrients. Ruminal ammonia concentration and protozoa number were not affected by the addition of ORE and CAR, which is in agreement with the lack of change in branched-chain VFA (i.e., sum of isobutyrate and isovalerate) molar proportion. Other studies also reported no effect of diet supplementation with leaves or extract on ruminal ammonia concentration (Hristov et al., 2013; Kolling et al., 2018). In contrast, Tekippe et al. (2011) observed a significant increase in ruminal ammonia concentration, a finding that contradicts the reported antimicrobial properties of oregano oil and carvacrol. Oregano oil and carvacrol have been shown to decrease protein degradation and ammonia concentration in vitro (Busquet et al., 2005; Macheboeuf et al.,

Table 6. Milk fat composition (g/100 g) of lactating cows fed a TMR without supplementation (control, CTL), or supplemented with monensin (MON), oregano oil (ORE), or carvacrol (CAR)

Item	Treatment				SEM	P-value
	CTL	MON	ORE	CAR		
4:0	3.450	3.367	3.341	3.464	0.051	0.27
6:0	1.963	1.864	1.928	1.929	0.027	0.11
8:0	1.130	1.048	1.114	1.098	0.022	0.08
10:0	2.795	2.541	2.735	2.657	0.077	0.15
12:0	3.384	3.070	3.342	3.204	0.110	0.21
14:0	11.240	10.712	11.289	10.910	0.238	0.30
<i>cis</i> -9 14:1	0.859	0.842	0.871	0.842	0.028	0.85
15:0	1.118	1.161	1.158	1.100	0.041	0.66
16:0	28.708	29.530	29.508	28.848	0.443	0.44
<i>cis</i> -9 16:1	1.071	1.182	1.079	1.144	0.040	0.19
<i>trans</i> -9 16:1	0.040	0.039	0.037	0.036	0.001	0.15
17:0	0.477 ^b	0.515 ^a	0.484 ^{ab}	0.491 ^{ab}	0.009	0.05
18:0	9.010	8.698	8.739	9.269	0.308	0.54
<i>cis</i> -9 18:1	15.269	15.784	14.840	15.593	0.524	0.61
<i>cis</i> -11 18:1	0.449	0.461	0.397	0.465	0.037	0.56
<i>cis</i> -12 18:1	0.247	0.259	0.256	0.246	0.007	0.46
<i>cis</i> -13 18:1	0.052	0.057	0.045	0.059	0.007	0.47
<i>trans</i> -5 18:1	0.017	0.017	0.017	0.018	0.001	0.66
<i>trans</i> -6–8 18:1	0.234	0.238	0.229	0.231	0.004	0.56
<i>trans</i> -9 18:1	0.167 ^b	0.179 ^a	0.169 ^b	0.166 ^b	0.003	0.01
<i>trans</i> -10 18:1	0.263 ^b	0.322 ^a	0.263 ^b	0.263 ^b	0.012	<0.01
<i>trans</i> -11 18:1	0.734 ^{ab}	0.707 ^b	0.793 ^a	0.718 ^{ab}	0.021	0.05
<i>trans</i> -12 18:1	0.359 ^b	0.397 ^a	0.366 ^{ab}	0.347 ^b	0.009	0.01
<i>trans</i> -15 18:1	0.362 ^b	0.409 ^a	0.365 ^b	0.361 ^b	0.007	<0.01
<i>trans</i> -16 18:1	0.292 ^b	0.336 ^a	0.286 ^b	0.292 ^b	0.008	<0.01
<i>cis</i> -15 18:1 + 19:0	0.074	0.068	0.075	0.076	0.002	0.15
<i>cis</i> -9, <i>cis</i> -12 18:2	1.911	1.914	1.945	1.910	0.031	0.83
<i>cis</i> -9, <i>trans</i> -11 18:2	0.321 ^{ab}	0.322 ^{ab}	0.354 ^a	0.304 ^b	0.012	0.05
<i>cis</i> -9, <i>trans</i> -12 18:2	0.028	0.031	0.027	0.027	0.002	0.31
<i>trans</i> -8, <i>cis</i> -13 18:2	0.066 ^b	0.081 ^a	0.067 ^b	0.066 ^b	0.003	<0.01
<i>trans</i> -9, <i>cis</i> -12 18:2	0.022	0.020	0.022	0.020	0.001	0.25
<i>trans</i> -9, <i>trans</i> -12 18:2	0.013	0.011	0.012	0.011	0.001	0.24
<i>trans</i> -10, <i>cis</i> -12 18:2	0.022	0.021	0.023	0.023	0.001	0.59
<i>trans</i> -11, <i>cis</i> -15 18:2	0.053	0.058	0.057	0.050	0.003	0.21
<i>trans</i> -9, <i>cis</i> -13 + <i>trans</i> -8, <i>cis</i> -12 18:2	0.258 ^{ab}	0.286 ^a	0.256 ^b	0.243 ^b	0.008	0.01
<i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12 18:3	0.027	0.025	0.027	0.024	0.001	0.25
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.450	0.459	0.453	0.453	0.013	0.97
<i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:4	0.014	0.017	0.014	0.016	0.001	0.29
20:0	0.130	0.126	0.132	0.133	0.002	0.27
<i>cis</i> -11 20:1	0.026	0.029	0.025	0.028	0.002	0.63
<i>cis</i> -11, <i>cis</i> -14 20:2	0.025	0.026	0.027	0.028	0.001	0.26
<i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14 20:3	0.090	0.081	0.088	0.088	0.004	0.36
<i>cis</i> -11, <i>cis</i> -14, <i>cis</i> -17 20:3	0.008	0.009	0.008	0.009	0.001	0.43
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14 20:4	0.110	0.105	0.106	0.106	0.002	0.48
<i>cis</i> -5, <i>cis</i> -8, <i>cis</i> -11, <i>cis</i> -14, <i>cis</i> -17 20:5	0.041	0.039	0.041	0.042	0.001	0.28
22:0	0.040	0.039	0.044	0.042	0.001	0.12
<i>cis</i> -13 22:1	0.006	0.006	0.006	0.006	0.0005	0.84
<i>cis</i> -7, <i>cis</i> -10, <i>cis</i> -13, <i>cis</i> -16 22:4	0.020	0.019	0.022	0.020	0.001	0.55
<i>cis</i> -4, <i>cis</i> -7, <i>cis</i> -10, <i>cis</i> -13, <i>cis</i> -16 22:5	0.009	0.010	0.010	0.011	0.001	0.29
<i>cis</i> -7, <i>cis</i> -10, <i>cis</i> -13, <i>cis</i> -16, <i>cis</i> -19 22:5	0.053	0.054	0.051	0.051	0.001	0.46
24:0	0.027	0.023	0.027	0.027	0.002	0.28
<i>trans</i> -11 18:1/ <i>trans</i> -10 18:1	2.800 ^a	2.241 ^b	3.023 ^a	2.801 ^a	0.136	<0.01
Glycerol	12.466	12.383	12.433	12.435	0.030	0.31

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

2008). The lack of effects of ORE and CAR on ruminal protozoa is in agreement with findings by Tekippe et al. (2011) and Hristov et al. (2013).

The only noticeable change in ruminal fermentation characteristics when MON was added to the diet was the increase in propionate molar proportion and, con-

sequently, a decrease in the acetate-to-propionate ratio. Monensin is known to shift rumen fermentation toward propionate production (Beauchemin et al., 2009; Martin et al., 2010). However, the effect of this ionophore on VFA pattern was inconsistent among studies and this discrepancy is more likely due to the dose, the

forage:concentrate ratio of the diet, or the source of starch, as discussed by Benchaar (2016).

Effect on Enteric Methane

Dry matter intake determined during the CH₄ measurement period (i.e., 7 d) averaged 23.2 kg/d (Table 4), which is close to average DMI (23.7 kg/d; on average) measured during the digestibility period (i.e., 7 d). Methane production averaged 491 g/d, 21.1 g/kg of DMI, and 6.14% of GE intake. These emissions are close to the emissions observed in our previous studies using respiration chambers for cows with similar levels of DMI (Benchaar et al., 2013; Hassanat et al., 2014).

Several in vitro studies have evaluated the antimethanogenic properties of oregano oil and carvacrol (see review by Benchaar and Greathead, 2011). However, information on the effect of oregano oil on enteric CH₄ production in dairy cows (i.e., in vivo) is very scarce and such information is not available for carvacrol. Using respiration chambers, Lejonklev et al. (2016) reported no effect on enteric CH₄ emission (L/d, L/kg DMI, or % of GE intake) in dairy cows fed oregano oil at 0.2 and 1.0 g/kg of DM (i.e., 4.3 and 22.4 g/d), which agrees with results presented in the current study. Kolling et al. (2018) evaluated the effects of oregano extract (0.056% of dietary DM) on enteric CH₄ production from dairy cows using respiration chambers. The authors reported no effect of the oregano extract on daily CH₄ emission (318 and 320 g/d, for the control and oregano extract treatments, respectively). When expressed on a DMI basis, CH₄ production tended ($P = 0.06$) to decrease in cows fed oregano extract compared with cows fed the control diet (15.3 vs. 19.7 g/kg of DMI). This decrease in CH₄ (g/kg of DMI) was mainly due to an increase in DMI measured in the chambers, for cows fed the oregano supplement compared with cows fed the control diet (20.6 vs. 16.3 kg/d; $P = 0.08$). It is worth noting that DMI measured outside of the chambers was unaffected by oregano extract. The decline in CH₄ yield (g/kg of DMI) observed by Kolling et al. (2018) was not supported by the lack of effects on rumen fermentation (i.e., pH, total VFA and acetate:propionate ratio) and nutrient (i.e., TDN) digestibility data. Therefore, it is possible that the reduction in CH₄ production was due to the increased DMI (+4 kg/d), which may have increased the ruminal passage rate. Previous studies (Okine et al., 1989; Hammond et al., 2014) showed that a faster ruminal passage rate is generally associated with a reduction in enteric CH₄ production (g/kg of DMI or % GE intake). More recently, Olijhoek et al. (2019) observed no change in enteric CH₄ production (g/d, g/kg of DMI, % of GE intake) in cows fed increasing amounts of dried whole-plant oregano with

either low (0.12% EO; 31% carvacrol; 23% thymol) or high (4.21% EO; 35% carvacrol; 41% thymol) levels of EO compared with cows fed the control diet. The absence of effect on CH₄ production in their study was consistent with the lack of changes in DMI, OM digestion (ruminal and total-tract), and rumen fermentation characteristics (i.e., acetate-to-propionate ratio).

Tekippe et al. (2011) and Hristov et al. (2013) evaluated the effect of including oregano leaves in dairy cow diets on CH₄ production using the SF₆ tracer technique and collecting gas samples from the rumen headspace at 2, 4, 6, and 8 h after feeding, during 1 d. In Tekippe et al. (2011), the oregano leaves supplement was introduced into the rumen as a pulse dose at the time of feeding. In that study, diet supplementation with 500 g of oregano leaves (7 g/d of EO; 6.9 g/d of carvacrol) decreased CH₄ production by 40% (expressed as g/h) but had no effect on DMI. In Hristov et al. (2013), dietary addition of oregano leaves (250, 500, and 700 g/d; 4.0, 7.9, and 11.9 g/d of EO; 3, 6, and 9 g/d of carvacrol, respectively), tended to decrease ($P = 0.06$) and increased linearly ($P = 0.05$) CH₄ production when expressed in grams per day and grams per kilogram of DMI, respectively. The greater decrease was observed with the medium level (i.e., 500 g/d) of oregano leaves inclusion (36 vs. 26% decrease) but not with the highest level (i.e., 750 g/d). It is possible that the inhibitory effect observed by Hristov et al. (2013) was due to the decrease in DMI when cows were supplemented with oregano leaves. In both studies, the decrease in CH₄ production was not consistent with the lack of effect on DMI (Tekippe et al., 2011), ruminal fermentation characteristics (pH, total VFA, and the acetate:propionate ratio), protozoa number, and *Methanobrevibacter*, the predominant methanogen in the rumen (Tekippe et al., 2011; Hristov et al., 2013). Several factors can explain this inconsistency. First, the validity of collecting gas samples from the rumen headspace versus breath samples. Second, the short duration of CH₄ measurement period (1 d; within 8 h after feeding), which makes uncertain that the effect of oregano leaves would have persisted over an entire 24 h. Benchaar et al. (2009) and Benchaar and Greathead (2011) reported the ability of rumen microbes to adapt to EO, degrade EO, or both. Third, in Tekippe et al. (2011), the oregano leaves were pulse-dosed into the rumen at the time of feeding, thus inducing a greater effect on CH₄ production during CH₄ measurement within 8 h of feeding. Because of these limitations in the method used by Tekippe et al. (2011) and Hristov et al. (2013) to quantify CH₄ emissions, the authors warned that CH₄ data from their studies must be interpreted with caution. Finally, as indicated by Tekippe et al. (2011), the concentrations of EO provided by 500 g/d of oregano leaves (i.e., 7.5 g/d of EO;

6.6 g/d of carvacrol) in their study are too high to feed to dairy cows under practical feeding conditions.

In the current study, compared with CTL, MON had no effect on CH₄ production. This result is consistent with the lack of MON effect on intake, ruminal digestion, and protozoa, but not with the lower acetate:propionate ratio. Reasons for this discrepancy are unclear because one of the mechanisms by which MON inhibits CH₄ production is by shifting rumen fermentation toward propionogenesis (Beauchemin et al., 2009; Martin et al., 2010).

Effect on Milk Production and Milk Composition

Supplementation of MON, ORE, and CAR did not affect milk production and milk composition. In agreement with Kolling et al. (2018), milk yield and milk composition were unaffected by feeding oregano extract to dairy cows (10 g/d; 0.0156% of dietary DM), results that were consistent with the absence of effects on DMI and ruminal fermentation characteristics in that study. Likewise, Hristov et al. (2013) reported no changes in milk yield and milk components in dairy cows fed increasing amount of oregano leaves (up to 750 g/d; 12 g/d EO; 9 g/d carvacrol). The lack of effect on milk yield was associated with a linear decrease in DMI. In a previous study by the same group (Tekippe et al., 2011), adding 500 g/d of oregano leaves (i.e., 7.0 g/d EO; 6.9 g/d carvacrol), did not affect milk yield and milk protein content but increased concentration and yield of milk fat. In Tekippe et al. (2011), DMI and the VFA profile (particularly the molar proportion of acetate) were not affected by oregano leaves supplementation, which does not explain the observed change in milk fat. Adding MON to the diet had no effect on milk yield and milk components, which agrees with other studies with cows fed MON at similar dose as used in our study (Grainger et al., 2010; do Prado et al., 2015; Benchaar, 2016).

Effect on Milk FA Composition

Little information is available on the effect of feeding oregano oil and carvacrol on milk FA profile. In the current study, no change in milk FA was observed when cows were fed ORE and CAR versus CTL. Likewise, other studies reported no or only minor effects on milk FA in dairy cows fed oregano leaves or extract (Hristov et al., 2013; Kolling et al., 2018). Benchaar (2016) reported no change in milk FA profile of cows fed the phenolic compound eugenol (50 mg/kg of dietary DM).

In the present study, feeding MON to cows affected the proportions of some FA in milk fat. One particular change of interest was the increase in the *trans*-10 18:1

proportion, whereas the *trans*-11 18:1 proportion was not changed. Consequently, the *trans*-11:*trans*-10 ratio was also decreased by MON supplementation. Using a continuous culture system of ruminal bacteria, Jenkins et al. (2003) observed that supplying monensin at 25 ppm increased the concentration of *trans*-10 18:1 but not that of *trans*-11 18:1. Increased milk fat concentration of *trans*-10 18:1 has been related to an inhibition of milk fat synthesis (Grinari et al., 1998). Benchaar et al. (2006) observed a higher concentration of *trans*-10 18:1 associated with lower milk fat content in cows fed MON. In the current study, such an increase in *trans*-10 18:1 was apparently not enough to induce inhibition of milk fat synthesis.

CONCLUSIONS

Results from this study do not confirm the antimicrobial activities of oregano oil and its main component, the phenolic carvacrol, as reported in several short-term in vitro studies. Under the conditions of this study, diet supplementation with ORE and CAR at 50 mg/kg of dietary DM did not affect nutrient utilization, ruminal fermentation characteristics, N excretion, CH₄ production, production, milk production, milk composition, or milk FA profile. The findings of enteric CH₄ production contrast with the limited in vivo data in the literature on feeding oregano plant, leaves, or extract to dairy cows. When supplied at 50 mg/kg of DM, oregano oil and carvacrol are likely not a viable strategy to improve feed efficiency, decrease N excretion, mitigate enteric methane, or enhance milk performance in dairy cows.

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