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Influence of Essential Oils Supplementation on Digestion, Rumen Fermentation, Rumen Microbial Populations and Productive Performance of Dairy Cows

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Abstract: Sixty lactating Holstein dairy cows were assigned to investigate the effect of Essential Oil Mixture (EOM) addition (mixture of eucalyptus oil 7%; menthol crystal 6.6% and mint oil 2.0%) on digestion, rumen fermentation, rumen microbial populations, milk production and milk composition. Cows were allotted into four groups (15 animals per each) and received EOM at (0, 16, 32 or 48 mg L^{-1} of drinking water) for successive eight weeks. Addition of EOM at 16 mg L^{-1} drinking water increased body weight gain, decreased feed intake and improved milk-to-feed ratio by about 7.4, 3.8 and 4.4%, respectively, across the whole experimental period when compared with the control, while moderate and higher addition levels of EOM (32 and 48 mg L^{-1} water) had variable results and not confirm the stability of animal performance. Addition of EOM had no significant effect on ruminal pH and ruminal fluid ammonia concentration and increased total Volatile Fatty Acid (VFA) by about 2.5, 2.9 and 0.7, respectively when compared with control. On the other hand, EOM (16 or 32 mg L^{-1} water) decreased molar proportion of acetate, whereas that of propionate increased compared with control and with the higher addition level (48 mg L^{-1}) of EOM receiving cows. Total viable bacteria, cellulolytic bacteria and protozoa counts were not changed with EOM supplementation. However, protozoa counts numerically decreased with EOM addition. Apparent digestibility of dry matter, organic matter and crude protein were slightly improved ($p>0.05$) with EOM supplementation compared with control. Addition of EOM (16 mg L^{-1}) improved ($p>0.05$) milk production across the whole experimental period, while the higher levels decreased ($p>0.05$) milk production when compared with control. Lower milk fat and higher milk protein was observed for cows received EOM than control and the milk protein showed the opposite direction. Results from this study suggest that EOM addition at 16 mg L^{-1} water slightly improved milk-to-feed ratio and productive performance and had limited effect on digestion and ruminal fermentation characteristics of dairy cows while, the higher dose may have negative effect of the productivity and ruminal fermentation of dairy cows.

Key words: Dairy cows, essential oils, rumen fermentation, nutrient digestibility, milk production

INTRODUCTION

There is no doubt that dietary antibiotics play a fundamental role in animal production as a growth and health promoter. However, the current trend is to look for alternatives to antibiotics because of public concerns as to their residues and subsequent occurrence of antibiotic-resistant

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bacteria. So that in recent years, public concern over the routine use of feed antibiotics and growth promoters in livestock production has increased. Accordingly, there is greater interest in using plant extracts as alternatives to antibiotics to manipulate ruminal fermentation and improve feed efficiency in ruminants (Hristov *et al*., 1999; Wang *et al*., 2000).

More recently, Essential Oils (EO) have attracted attention for their potential as alternatives to feed antibiotics and growth promoters in livestock (Wallace, 2004). Contrary to what their name might suggest, EO are not oils (i.e., lipids) because they consist solely of naturally occurring volatile components that can be extracted from plants by distillation methods, in particular steam distillation (Greathead, 2003). Chemically, EO is variable mixtures of principally terpenoids, especially monoterpens (C_{10}) and sesquiterpenes (C_{15}), although diterpenes (C_{20}) may also be presents. EO may also include a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, or lactones and exceptionally nitrogen and sulfur containing compounds, coumarins and homologs of phenylpropanoids (Dorman and Deans, 2000; Hummelbrunner and Isman, 2001).

Essential Oil (EO) from a variety of sources has been shown to alter the bacterial growth and metabolism of several types of bacteria, including rumen bacteria (Wallace, 2004). Recently, a number of studies have been published on the effects of EO on rumen microorganisms and rumen metabolism. Most of these studies are short term *in vitro* culture incubations (McInotch *et al*., 2003; Cardozo *et al*., 2004; Busqut *et al*., 2005; Castillejos *et al*., 2005) or *in situ* incubations (Benchaar *et al*., 2003, 2006b, 2007; Molero *et al*., 2004; Newbold *et al*., 2004) have been conducted *in vivo* to evaluate the influence of EO on ruminant metabolism and performance. Also a number of recent *in vitro* studies using batch or continuous cultures have evaluated effects of various EO and their compounds on ruminal microorganisms and ruminal metabolism and have reported varied results (Busquet *et al*., 2006; Castillejos *et al*., 2006; Fraser *et al*., 2007). Inconsistencies among studies may be attributed to factors such as the chemical composition of the EO, the concentration used and interaction among the bioactive agents in EO (Dorman and Deans, 2000), the *in vitro* technique used may also influence results.

The objective of the present study was to investigate the effects of water addition of a specific Essential Oil Mixture compounds (EOM) on digestion, ruminal fermentation characteristics, ruminal microbial populations, milk production and milk composition of dairy cows.

MATERIALS AND METHODS

This experiment was carried out at the Al-Ghadeer Dairy Farm in the Kingdom of Saudi Arabia (KSA) starting from mid September to mid November 2007.

Cows, Experimental Design and Diets

Sixty lactating Holstein dairy cows were allotted into four groups (15 cows per each). First group (15 cows) was assigned to the control (without any EO mixture product) and the other three cow groups received EO mixture product aeroforte (produced by Kanters Special products Co. (Netherlands) and contained (ethanol, 22.5%; emulsifiers, 15.3%; eucalyptus oil 7%; menthol crystal 6.6%; mint oil 2.0% and demineralized water up to 100%) at 0.0, 10.0, 20.0 and 30.0 mL/100 L of water which supplied 0.0,16, 32, 48 mg L^{-1} of drinking water for the four groups, respectively for 8 successive weeks. The cows averaged 84±12 day in production (DIM) at the start of the experiment, with an average Body Weight (BW) of 575 kg. They were housed in bedded pack housing system with outside feeding. The housing system provides 0.45 cm head lock for feeding and about 30 m^2 yard per cow. The barns have open ridges to facilitate cleaning and manure removing. About 30% of the whole area is sloppy shaded at height 9 m in the center with fan but no available cooling system in the cow's yard. Waters were available in suitable size all the day time. Cows were fed ad libitum on a Total

Mixed Ration (TMR) to meet the predicted requirements for energy, protein, minerals and vitamins according to NRC (2001). Ingredient and chemical compositions of the used diet are presented in Table 1.

Feed Intake, Water Consumption and Body Weight

Diets were offered in equal amounts twice daily (08:00 and 15:00). Feed consumption was recorded daily by weighing feeds offered to and refused by the cows. Water intake was also monitored using one cement tank for each group and the daily consumption was recorded by estimating the offered and refused water quantity. Samples of TMR and feed ingredients were collected daily and kept frozen. Samples were composited by period (each 4 weeks), dried at 55°C for 48 h, ground through a 1 mm screen (Wiley mill) and analyzed for DM, OM, total nitrogen, NDF, ADF, EE. Body Weight (BW) was determined at the beginning and at the end of each 28 days of the experimental period after the morning milking.

Apparent Total-Tract Digestibility and Nitrogen Balance

On 49 day of the experimental periods, feces samples were collected for 7 consecutive days, stored at -20°C and subsequently thawed, dried at 55°C for 48 h and ground through a 1 mm screen (Wiley mill) for chemical analysis. Apparent Digestibility Coefficients (ADC) of nutrients were determined using an internal marker Acid Insoluble Ash (AIA) in the diets. ADCs for the different nutrients are expressed as the fractional absorption of these nutrients from diets in the cow:

$$ADC\ nutrient = 1-(AIA_{diet}/AIA_{feces} \times nutrient_{feces}/nutrient\ diet) \times 100$$

where, AIA_{diet} is the AIA in the diet (%), AIA_{feces} is the AIA in the feces (%), $nutrient_{feces}$ is the nutrient in the feces (%) and $nutrient_{diet}$ is the nutrient in the diet (%). Total urine was collected daily into stainless steel containers and acidified with H_2SO_4 (50% v/v) to maintain pH<2.0. A representative sample (2%) was taken and kept frozen at -4°C until analysis.

Milk Production and Milk Composition

Cows were milked twice daily in the milking pallor (05:00 and 17:00) and milk yield was recorded at each milking. During the last week of each 28 day period, milk samples were taken from

Table 1: Ingredient composition (% as fed) and chemical composition (on dry matter basis) of the diet

Ingredient composition (%)		Chemical composition	
Ingredient	Values	Items	Values
Berseem hay	50.00	Crude protein (%)	16.40
Yellow corn	20.50	Ether extract (%)	3.50
Barley grain	15.35	Crude fiber (%)	16.80
Soybean meal	5.50	NDF (%)	28.10
Cotton seed	1.00	ADF (%)	20.70
Sugar beet pulp	1.90	Ash (%)	8.80
NaCl (Iodinized)	0.10	NE_L (MJ/kg DM)***	1.58
Dicalcium phosphate	0.50	Calcium (%)	1.05
Lime stone	1.00	Phosphorus (%)	0.40
Sodium bicarbonate	0.30		
Rumen protected fat*	1.25		
Mineral and vitamin premix**	0.10		
Molasses	2.50		

* Super Sp-202, produced by All green Co. (Malaysia) a special form of a hydrogenated triglyceride which has min. 99% fat as palm oil, acid value, 220 max, melting point, 55°C min. ** Cattle premix produced by Centraly's Co. (France) contains the following elements per kg, (10000000 IU vitamin A, 1000000, IU vitamin D_3, 10000 mg vitamin E, 100000 mg magnesium, 50000 mg manganese, 45000 mg zinc, 50000 mg iron, 6000 mg copper, 800 mg iodine, 100 mg selenium. *** Calculated according to NRC (2001)

five cows of each group at each milking, pooled on a yield basis and stored at -4°C with a preservative (bronopol-B2) until analyzed for fat, protein and lactose.

Rumen Fermentation Characteristics

Ruminal fluids were collected from 5 dairy cows at 0, 4 and 8 h after feeding from 5 cows from each group during the 4th and 8th weeks of the experiment. Ruminal fluids collected through a speculum were inserted into the cow mouth and a lubricated rubber tube was inserted through the speculum into the rumen via., the esophagus. Ruminal contents (250 mL) were removed using an electric pump. Samples were monitored visually to ensure they were not contaminated with saliva. The pH was measured immediately using pH meter (Orion research model 201). The whole contents were squeezed through 4 layers of cheesecloth. The samples were acidified to pH 2 with 50% H_2SO_4 and frozen at -20°C for later determination of Volatile Fatty Acids (VFA) and ammonia (NH_3-N) concentrations.

Microbial Counts

Microbial counts were carried out on ruminal fluid samples collected 4 h after the am feeding. Ruminal fluid (L^{-1}) and solid digesta (500 g) samples were collected from the rumen of five cows from each group. Samples were mixed thoroughly and subsamples of 500 mL of ruminal fluids and 250 g of solid digesta were blended anaerobically under oxygen-free CO_2 and strained through 2 layers of cheesecloth. A 3 mL portion of the strained ruminal fluid was preserved using 3 mL of methyl green formalin-saline solution for protozoa enumeration (Ogimoto and Imai, 1981). Protozoa samples were stored at room temperature in the dark until counting. Protozoa were enumerated microscopically in a Levy-Hausser counting chamber (Hausser Scientific, Horsham, PA). Each sample was counted twice and if the average of the duplicates differed by more than 10%, the counts were repeated.

Serial 10 fold dilutions of strained ruminal fluid were prepared under 95% CO_2, 5% H_2 in an anaerobic chamber and used as inoculum for microbial counts (Bryant and Burkey, 1953). Total viable counts were enumerated on triplicate layered plates (Koch, 1994) containing ruminal fluid-starch-agar medium (Grubb and Dehority, 1976). Cellulolytic bacteria were counted by the most probable method based on the degradation of a filter paper strip (Mann, 1968).

Chemical Analysis

Analytical DM contents of TMR, feed ingredients and feces were determined by oven-drying at 105°C for 48 h (AOAC, 1990; method 930.15). Ash contents of TMR, feed ingredients and feces were determined by incineration at 550°C overnight and the OM content was calculated as the difference between 100 and the percentage of ash (AOAC, 1990; method 942.05). The concentration of nitrogen in acidified urine samples was determined by micro Kjeldahl analysis (AOAC, 1990).

Feed and feces samples were analyzed for Acid Insoluble Ash (AIA) content by dissolving the obtained ash in hydrochloric acid following the ISO 5985 (1998) procedures. Crude protein in TMR, feed ingredient and feces were determined by using Kjeldahl method according to Randhir and Pradhan (1981) and ether extract was determined according to Bligh and Dyer (1959) technique as modified by Hanson and Olly (1963). NDF and ADF in TMR and feed ingredients were determined according to (AOAC, 1990; method 973.18). Protein, fat and lactose concentrations in milk samples were analyzed (AOAC, 1990) by infrared spectrophotometer (System 6000 MilkoScan; Foss Electric). Concentrations of NH_3-N and VFA in ruminal fluid were analyzed by colorimetry (Weatherburn, 1967) and by GLC (Varian 3700; Varian Specialties Ltd., Brockville, Ontario, Canada), respectively.

Statistical Analysis

Data were analyzed by the General Linear Model (GLM) procedure (SAS, 1996). The Least Square Mean (LSM) ± standard errors were calculated and tested for significance using the t-test (Steel and Torrie, 1960).

RESULTS AND DISCUSSIONS

Body Weight, Feed Intake and Water Consumption

Initial BW averaged 575 kg and was similar among treatments (Table 2). However, final BW was significantly higher ($p<0.05$) for cows received Essential Oil Mixture EOM (16 or 32 mg L^{-1} drinking water) than those received no EOM or received the higher dose of EOM. In regard to the average daily BW gain across the whole experimental periods it was observed that higher BW gain in cows received the lower and moderate dose of EOM when compared with the control (0.4, 0.35 vs 0.23, respectively) while, higher EOM inclusion level had negative effect (0.2 vs 0.23 kg day^{-1}, respectively). The present results are supported by Benchaar *et al*. (2006b) stated that BW gain was higher for cows fed EO than for those fed no EO (0.44 vs 0.15 kg day^{-1}, respectively). Moreover, statistical analysis revealed that there was no significant increase in the animal BW during 1st and 2nd month of the experiment for animal in the control or received 48 mg EOM L^{-1} of drinking water, while significantly improved with 16 or 32 mg EOM L^{-1} of drinking water.

Addition of EOM decreased DMI when was expressed in kilograms per day or when expressed as a percentage of BW (Table 3). EOM addition at (16, 32 or 48 mg L^{-1} of drinking water) lowered ($p>0.05$) DMI across the whole experimental period by about 3.8, 3.8 and 2.2%, respectively when compared with control. It was clear EOM addition had no significant effect on DMI between the 1st week of the experiment and 2nd month of the same group except of the cows received the lower level of EOM in the drinking water recorded a significant reduction of DMI during the 2nd month when compared with the 1st month of the experiment. The trend of lower DMI is in agreement to results of Benchaar *et al*. (2006b) who reported that dairy cattle fed on EO treated diet had lower DMI when compared with control. While, the results is in contrast to Benchaar *et al*. (2003) who observed no change in DMI when lactating dairy cows were fed a mixture of EO (750 mg day^{-1}), whereas feeding greater amounts (2 and 4 g/head /day) of a blend of EO compounds (include thymole, eugenol, vanillin and limonene) increased DMI of growing beef cattle fed silage based diets (Benchaar *et al*., 2006a). Tedeschi *et al*. (2003) speculated that the variability between studies is probably due to differences in the stage of lactation and that a reduction in DMI is a consequence of animal eating to their energy requirement. When cows are in positive energy balance (late lactation

Table 2: Effect of essential oil mixture supplementation on body weight changes (kg cow^{-1}) of dairy cows

	EOM mg L^{-1} of drinking water (n = 15)			
Stage of reproduction cycle	0	16	32	48
12 weeks postpartum (initial)	572±3.3^{ax}	576±3.0^{ax}	576±3.6^{ax}	573±3.0^{ax}
16 weeks postpartum	579±3.3^{bxz}	588±2.6^{ay}	586±3.3^{aby}	578±3.0^{bxz}
20 weeks postpartum (final)	586±3.5^{byz}	598±2.4^{az}	596±3.3^{az}	579±2.8^{byz}
Change (kg day^{-1})	0.23±0.02^{b}	0.4±0.03^{a}	0.35±0.02^{a}	0.20±0.0^{b}

n: No. of observation; Means±SE. $^{a-b}$ Means within the same row having different letters are significant different ($p\leq0.05$). $^{x-z}$ Means within the same column having different letters are significant different ($p\leq0.05$)

Table 3: Effect of essential oil mixture supplementation on dry matter intake (kg/cow/day) of dairy cows

	EOM mg L^{-1} of drinking water							
	0		16		32		48	
Stage of lactation	kg day^{-1}	% from BW	kg day^{-1}	% from BW	kg day^{-1}	% from BW	kg day^{-1}	% from BW
13-16 weeks	18.1±0.1^{a}	3.1	17.7±0.1^{ax}	3.0	17.5±0.1^{a}	3.0	17.7±0.1^{a}	3.1
17-20 weeks	18.3±0.1^{a}	3.1	17.3±0.1^{ay}	2.9	17.5±0.1^{a}	2.9	17.8±0.1^{a}	3.1
Average	18.2±0.1	3.1	17.5±0.1	3.0	17.5±0.1	3.0	17.8±0.1	3.1

Means±SE. $^{a-b}$ Means within the same row having different letters are significant different ($p\leq0.05$). $^{x-y}$ Means within the same column having different letters are significant different ($p\leq0.05$)

or dry cows), supplementation of EOM may increase the energy available per unit of feed consumed (Mcal day^{-1}), thus resulting in lower DMI. On the other hand, when cows are in negative energy balance (early lactation) the additional energy available due to EOM addition is used to improve performance, reduce body reserve losses (Tedeschi *et al*., 2003). Cows used in the present experiment have a positive energy balance as presented in Table 5.

EOM addition increased water intake across the whole experimental period by about 1.7, 5.8 and 9.2%, respectively when compared with control (Table 4). There are no earlier reports on the effects of EOM (eucalyptus oil, menthol crystal and mint oil) on water consumption in lactating cows. However, there is evidence that capsaicin, the active component of capsicum oil, increases water intake in rats (Zafra *et al*., 2003). Also, Cardozo *et al*. (2006) indicated that capsicum extract increased ($p<0.05$) water intake in growing heifers compared with control while a reduction in water intake of growing heifers fed on anise oil and mixture of cinnamaldehyde and eugenol were observed. Water consumption confirmed that cows received about 0.0, 1872, 3963 and 6131 mg of EOM/cow/day for the four groups, respectively.

Rumen Fermentation Characteristic

Table 5 shows changes in the pH value, ammonia and VFA production caused by the addition of EOM compared with the control. The mean pH value tended to be slightly lower in the ruminal fluid of cows received water treated with EOM (16, 32 or 48 mg L^{-1}) as compared with control (6.42, 6.35, 6.39 vs 6.44, respectively). Tatsouka *et al*. (2008) stated that addition of eucalyptus oil to ruminal fluid during in vitro incubation reduced the pH value. These data is in contrast with the results of Benchaar *et al*. (2006b) who reported higher ruminal pH in cows fed diets supplemented with 2 g day^{-1} of Crina ruminants (blend of thymol, eugenol, vanillin and limonene) than in cows fed diets without supplementation. Evans and Martin (2000) also reported that the addition of 400 mg L^{-1} of thymol increased the pH in 24 h *in vitro* batch culture of mixed rumen bacteria, but no effects were reported at lower doses (50, 100 and 200 mg L^{-1}). The variable results may be related to the type of EOM and ration fed to the animals as well as the length of the adaptation period.

Addition of EOM in the drinking water had no effect on the ruminal fluid concentration of NH_3-N (Table 5). These data are in contrast with the short term *in vitro* results that

Table 4: Effect of essential oil mixture supplementation on water consumption (L/cow/day) of dairy cows

	EOM mg L^{-1} of drinking water			
Stage of reproduction cycle	0	16	32	48
13-16 weeks	121±2.9^{a}	119±3.7^{a}	125±3.7^{a}	129±4.5^{a}
17-20 weeks	118±4.2^{b}	124±3.5^{ab}	128±4.9^{ab}	132±4.8^{a}
Average (L/cow/day)	120±3.6^{b}	122±3.5^{b}	127±4.2^{ab}	131±4.9^{a}
Relative to control	100	+ 1.7	+ 5.8%	+ 9.2 %
Average EOM intake (mg/cow/day)	0	1872	3963	6131

Means±SE. $^{a-b}$ Means within the same row having different letters are significant different ($p \leq 0.05$)

Table 5: Effect of essential oil mixture supplementation on rumen fermentation characteristics in dairy cows

	EOM mg L^{-1} of drinking water (n = 10)			
Item	0	16	32	48
pH	6.44±0.03^{a}	6.42±0.02^{a}	6.35±0.02^{a}	6.39±0.01^{a}
NH_3-N (mg/100 mL)	9.56±0.10^{a}	9.66±0.10^{a}	9.52±0.20^{a}	9.46±0.20^{a}
Total VFA (mM)	96.40±1.40^{b}	98.80±1.50^{ab}	99.20±1.80^{a}	97.15±1.30^{b}
VFA mol/100 mL Acetate (A)	65.20±0.40^{a}	61.10±0.90^{b}	60.80±0.90^{b}	64.30±1.10^{a}
Propionate (P)	20.80±0.40^{b}	23.70±0.60^{a}	23.60±0.50^{a}	21.30±0.50^{b}
Butyrate	13.90±0.70^{ab}	15.20±1.10^{ab}	16.30±1.10^{a}	14.40±0.90^{ab}
A:P ratio	3.13±0.10^{b}	2.63±0.10^{c}	2.55±0.10^{c}	4.45±0.10^{a}

n: No. of observation; Means±SE. $^{a-b}$ Means within the same row having different letters are significant different ($p \leq 0.05$)

indicated that lower NH_3-N concentration in the ruminal fluid (McInotch *et al*., 2003; Newbold *et al*., 2004). On the other hand, longer term *in vitro* incubations (Castillejos *et al*., 2005) and *in vivo* (Newbold *et al*., 2004; Benchaar *et al*., 2006b, 2007) reported no change in ruminal fluid NH_3-N concentration when compared with control. This discrepancy among studies could be due to the difference of the experimental procedure used. The greater exposure time of ruminal bacteria to essential oils may allow ruminal microbes to adaptation as shown by (Cardozo *et al*., 2004; Busqut *et al*., 2005), who observed that some of the effect of essential oils on ruminal fermentation disappeared after 6 to 7 days of fermentation in a dual-flow continuous culture system, indicating that rumen microbes are able to adapt to essential oils and more research is required to understand the mechanisms of rumen microorganisms adaptation to essential oil compounds.

Addition of EOM (16, 32 and 48 mg L^{-1} of drinking water per head/day) increased the ruminal total VFA concentration by about 2.5, 2.9 and 0.7%, respectively when compared with the control. Molar proportions of individual VFA and the acetate to propionate ratio were significantly ($p<0.05$) affected by the lower and medium dose of EOM addition as increased the propionate proportion and decrease the acetate and consequently decrease the acetate to propionate ratio compared with control, however, the higher addition level of EOM had non significant effect on those fermentation parameters. The present data are in agreement with Castillejos *et al*. (2005) who observed an increase in the total VFA concentration but no change in molar proportions of individual VFA when Crina ruminants EOM (thymol, eugenol, vanillin and limonene) was added at a dose of 1.5 mg L^{-1} of ruminal fluid culture in continuous culture fermenters. More recently, *in vitro* ruminal culture (Tatsuoka *et al*., 2008) reported that eucalyptus increased total VFA and proportion of propionic acid concentration compared with the control.

In the present study, the molar proportion of acetate decreased, whereas that of propionate increased (group 2 and 3) compared with control and the higher EOM addition (group 4). As a result, the acetate to propionate ratio was lower which may contribute to explaining the slightly lower milk fat and the little higher milk protein concentrations (Table 8) for cow received 16 or 32 mg of EOM L^{-1} water than those with control.

Rumen Bacterial Counts

Data on the effects of essential oils on ruminal microbial populations are scarce. In the present experiment, total viable bacteria, cellulolytic bacteria and protozoa counts were not changed by addition of EOM in the drinking water (Table 6). However, protozoa counts numerically decreased with EOM addition (16, 32 or 48 mg L^{-1} water) by about 4.4, 5.2 and 6.6%, respectively when compared with control and that indicate the reduction possibility of methane production in the rumen of the dairy cattle with EOM addition based on eucalyptus, menthol and mint oils due to 9-25% of methane production in the rumen could be attributed to protozoa associated methanogenic bacteria (Tatsuoka *et al*., 2008). Other studies (Benchaar *et al*., 2003, 2006b, 2007; Newbold *et al*., 2004) reported no effect of essential oil on the number and composition of the ciliate protozoal populations. In regards to the total viable bacteria and cellulolytic bacteria counts these results supported by Wallace *et al*. (2002) who observed no changes in total viable bacteria counts in sheep fed high or low protein diets supplemented with 100 mg day^{-1} of Crina ruminants MEO.

Total Tract Nutrient Digestibilities and Nitrogen Balance (N Balance)

Apparent digestibilities of DM, OM and CP as affected by EOM addition are presented in (Table 7). EOM supplementation (16, 32 or 48 mg L^{-1} water per head/day) increased ($p>0.05$) DM digestibility by about 0.9, 0.9 and 1.4%, respectively when compared with control. Moreover, also both OM and CP digestibilities were improved ($p>0.05$) with different addition levels of EOM in the drinking water for dairy cattle when compared with control. The present data are supported by the

Table 6: Effect of essential oil mixture supplementation on rumen microbial counts in dairy cows

	EOM mg L^{-1} of drinking water (n = 10)			
Item	0	16	32	48
Total viable bacteria, $\times 10^9$ mL^{-1}	2.44±0.05[a]	2.50±0.07[a]	2.46±0.04[a]	2.54±0.05[a]
Cellulolytic bacteria, $\times 10^7$ mL^{-1}	3.26±0.05[a]	3.32±0.07[a]	3.28±0.08[a]	3.20±0.04[a]
Protozoa, $\times 10^5$ mL^{-1}	4.98±0.05[a]	4.76±0.08[a]	4.72±0.07[a]	4.65±0.07[a]

n: No of observation; Means±SE. [a-b] Means within the same row having different letters are significant different ($p \leq 0.05$)

Table 7: Effect of essential oil mixture supplementation on total tract apparent nutrient digestibility and nitrogen balance in dairy cows

	EOM mg L^{-1} of drinking water (n = 14)			
Item	0	16	32	48
Dry matter digestibility (%)	65.6±0.4[a]	6.2±0.4[a]	66.2±0.5[a]	66.5±0.6[a]
Organic matter digestibility(%)	67.9±0.3[a]	68.9±0.2[a]	68.9±0.3[a]	68.6±0.5[a]
Crude protein digestibility(%)	62.4±0.5[a]	63.9±0.5[a]	63.2±0.8[a]	64.9±0.7[a]
Nitrogen balance (g day^{-1})				
Intake	477	459	459	467
Feces	179	166	167	164
Milk	143	150	146	144
Urine	121	108	115	129
Retained	+34	+35	+31	+30

n = No. of observation; Means±SE. [a-b] Means within the same row having different letters are significant different ($p \leq 0.05$)

results obtained by Benchaar *et al*. (2006b) who reported no change in apparent total tract digestibilities of DM, CP and NDF in lactating cows supplemented with 2 g day^{-1} of Crina ruminants EOM. Outputs of N in feces, urine and milk were not influenced by EOM supplementation resulting in a nearly similar retention of N between EOM supplementation and control cows. However, N retention numerically higher in the cows received 16 mg of EOM L^{-1} water when compared with the control and other addition levels of EOM. The present data is in contrast with that obtained by Benchaar *et al*. (2006b) who observed no change in N retention when cows fed 2 g day^{-1} of the Crina ruminants (Mixture of essential oil) supplement.

Milk Production and Milk Composition

Production of milk (averaged 28.9 kg day^{-1}) and was not affected by additive (Table 8). The present results revealed that milk production nearly similar between different groups during the first month while slightly ($p>0.05$) increased across the whole experiment with EOM (16 mg L^{-1} water) by about 1% when compared with control and decrease ($p>0.05$) with the other dose of EOM by about 1.0 and 1.4%, respectively. Moreover, it was observed that there was a significant decrease in milk production during the 2nd month of the experimental period of the control dairy cows or that received 32 and 48 mg EOM L^{-1} of drinking water when compared with the production during the 1st month of the same group, while the dairy cows received lower addition level of EOM recorded non significant improvement of milk production during the 2nd experimental period when compared with the same group, while the dairy cows received lower addition level of EOM recorded non significant improvement of milk production during the 2nd experimental period when compared with the same group during the 1st month. However, EOM supplementation (16, 32 or 48 mg L^{-1} water) non significantly lowered fat percent by about 2.5, 4.4 and 2.2%, respectively when compared with control while protein percent followed an opposite trend as it increased ($p>0.05$) with EOM supplementation by about 5.1, 5.5 and 3.5%, respectively. On the other hand fat and protein yield followed the same trend of its percentage in the milk. Moreover, EOM addition raised milk lactose concentration by about 2.6, 1.3 and 1.7%, respectively when compared with control. These data are in agreement with Benchaar *et al*. (2006b) who observed that essential oil had no effect on milk production but the yield

Table 8: Effect of essential oil mixture supplementation on milk production (kg/cow/day) and composition in dairy cows

	EOM mg L^{-1} of drinking water (n = 14)			
Item	0	16	32	48
Milk production (kg day^{-1})				
13-16 weeks	29.70±0.20^{ax}	29.30±0.20^{ax}	28.90±0.30^{ax}	29.40±0.20^{ax}
17-20 weeks	28.50±0.20^{ay}	29.04±0.20^{ax}	28.70±0.20^{ay}	27.90±0.20^{ay}
Average production	29.10±0.20^{a}	29.40±0.20^{a}	28.80±0.20^{a}	28.70±0.20^{a}
Milk composition (%)				
Fat	3.19±0.03^{a}	3.11±0.04^{a}	3.05±0.01^{a}	3.12±0.02^{a}
Protein	3.11±0.02^{b}	3.27±0.05^{a}	3.28±0.03^{a}	3.22±0.03^{a}
Lactose	4.60±0.50^{a}	4.72±0.04^{a}	4.66±0.03^{a}	4.68±0.04^{a}
Milk constituents yield (kg day^{-1})				
Fat	0.91±0.01^{a}	0.91±0.01^{a}	0.88±0.01^{a}	0.89±0.01^{a}
Protein	0.91±0.01^{b}	0.96±0.02^{a}	0.93±0.01^{ab}	0.92±0.01^{b}
Lactose	1.34±0.01^{a}	1.39±0.01^{a}	1.32±0.01^{a}	1.33±0.01^{a}

n = No. of observation; Means±SE. ^{a,b} Means within the same row having different letters are significant different ($p \leq 0.05$). ^{x,y} Means within the same column having different letters are significant different ($p \leq 0.05$)

Table 9: Effect of essential oil mixture supplementation on feed conversion efficiency and Net Energy Balance (NEB) of lactating cows

	EOM mg L^{-1} of drinking water (n = 28)			
Item	0	16	32	48
Milk-to-feed ratio				
13-16 weeks	1.64±0.01^{ax}	1.66±0.02^{ax}	1.65±0.02^{ax}	1.63±0.02^{ax}
17-20 weeks	1.56±0.01^{by}	1.67±0.02^{ax}	1.57±0.01^{by}	1.57±0.01^{by}
Average (13-20 weeks)	1.60±0.01^{b}	1.67±0.01^{a}	1.61±0.01^{ab}	1.60±0.01^{b}
Net energy balance*	12.60	11.20	10.70	12.20

n = No. of observation; Means±SE. ^{a,b} Means within the same row having different letters are significant different ($p \leq 0.05$). * Net energy balance (calculated based on NRC, 2001) = (Dry matter intake x NE_L content of diet)-{(0.08 x body $weight^{0.75}$) + ((0.0929 x fat% + 0.0563 x protein% + 0.0395 x lactose) x milk yield)}

of 4% fat corrected milk decreased. Also Benchaar *et al*. (2007) observed that feeding cows on mixture of essential oil treated diets had no effect on milk concentrations of fat, protein and milk yields of fat and protein while, tended to be increased milk lactose concentrations when compared with cow fed no essential oil. On the other hand the lower fat percent produced by cows received EOM is in contrary with reported by (Yang *et al*., 2007) who observed that milk fat was higher for garlic essential oil addition (3.46%) and berry essential oil addition (3.4%) when compared with the control (3.14%) and the difference may be due to the present study slightly decreased acetate and increased propionate concentration of the rumen VFA.

Feed Efficiency

EOM water addition had no effect on the milk-to-feed ratio during the first month of the experimental period, while there was a non-significant tendency within the 2nd that it was improved ($p>0.05$) with EOM supplementation at 16 mg L^{-1} drinking water by about 7.1% when compared with the control group (Table 9). On the other hand it was observed that cows received higher dose of EOM supplementation (32 and 48 mg L^{-1} water per head/day) showed the same milk-to-feed ratio as the control which explain that the effect of EOM are related to the time and dose of usage. On the average of the entire period milk-to-feed ratio was improved by 4.4, 0.6 and 0.0%, respectively when compared with control.

Cows receiving EOM had a less positive net energy balance across the whole experimental period by about (11, 15 and 3.2%, respectively) than the untreated cows. This trend of net energy balance reduction may be related to the lower feed intake with EOM supplementation.

CONCLUSION

It could be concluded that EOM addition (16 mg L^{-1} water per head/day) may be useful as additives as it improved daily gain, nutrient digestibility, milk-to-feed ratio and milk production of dairy cows. Moreover, EOM addition appear to be reduced dry matter intake, considering that increasing total VFA, reducing acetate and increasing propionate concentrations is more efficient for dairy cattle production. On the other hand higher addition levels for long term feeding period may be have an adverse effect of the metabolism and productivity of dairy cows.

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