

Effect of a mixture of supplemental dietary plant essential oils on performance of periparturient and early lactation dairy cows

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ABSTRACT

Plant essential plant oils (EO) are volatile aromatic compounds with antimicrobial activity that can alter ruminal fermentation when used as dietary supplements. A feeding trial was conducted to determine the effects of dietary supplementation of periparturient and early lactation dairy cows with a specific mixture of EO. Forty multiparous Holstein cows were randomly assigned to either control (C) or EO-supplemented (1.2 g/cow per day) total mixed rations (TMR). Feeding of treatment diets commenced 3 wk before the expected calving date and continued through 15 wk in lactation. The prepartum TMR contained 70% forage [70% corn silage, 15% alfalfa silage, and 15% wheat straw; dry matter (DM) basis]. The lactation TMR contained 50% forage (60% corn silage, 33% alfalfa silage, 7% alfalfa hay; DM basis). Prepartum and lactation TMR were formulated to contain 12 and 17% CP (DM basis), respectively. There were no differences between treatments for prepartum DM intake (DMI), but DMI was 1.8 kg/d less for EO than C on average across the 15-wk lactation trial. Plasma concentrations of glucose, nonesterified fatty acids, β -hydroxybutyrate, and urea-N on samples collected -21, -14, -7, -1, 1, 8, 15, 22, and 29 d relative to calving were unaffected by treatment. There were no differences between treatments for actual or fat-corrected milk yields on average across the 15-wk lactation trial. Milk protein content was 0.15% units less for EO than C. Feed efficiency (kg of milk per kg of DMI) tended to be greater for EO than C on average and was greater during wk 8 to 14 of lactation. Prepartum and lactation body weight and condition score measurements were unaffected by treatment. There was no benefit to EO in prepartum dairy cows. Dietary supplementation with EO reduced DMI in early lactation dairy cows with no effect on milk yield.

Key words: essential oil, intake, lactating cow, periparturient cow

INTRODUCTION

Essential oils (EO) are volatile aromatic compounds with an oily appearance extracted from plants and are secondary metabolites usually made up of terpenoids and phenylpropanoids (Calsamiglia et al., 2007). Plant EO exhibit a wide range of antimicrobial activities (Burt, 2004) and have gained interest as a possible natural replacement for antibiotic rumen fermentation modifiers due to the increase in public concern over antibiotic residues and resistance. Some of the more common EO compounds include (Calsamiglia et al., 2007): thymol (thyme and oregano), eugenol (clove), pinene (juniper), limonene (dill), cinnamaldehyde (cinnamon), capsaicin (hot peppers), terpinene (tea tree), allicin (garlic), and anethol (anise).

Calsamiglia et al. (2007) from an extensive review of the in vitro, in situ, and continuous culture-based literature concluded the following about the ruminal effects of EO: 1) inhibition of deamination and methanogenesis resulting in lower $\text{NH}_3\text{-N}$, methane, and acetate and higher propionate and butyrate concentrations; 2) variable responses depending on the specific EO or combination of EO supplemented; and 3) effects of some EO are pH- and diet-dependent. Increases in ruminal true OM and N digestibilities (Yang et al., 2007), total tract ADF and starch digestibilities (Benchaar et al., 2006), and DMI, milk yield, and fat yield (Kung et al., 2008) in dairy cows have been reported for EO.

Although experiments have been done to evaluate the effect of EO in lactating dairy cows, trials using transition cows and early lactation cows, or both, are lacking in the literature. The objective of the experiment reported herein was to determine the effect of a specific mixture of plant EO on DMI, milk yield and composition, and blood parameters when fed to periparturient and early lactation dairy cows.

MATERIALS AND METHODS

Forty multiparous Holstein cows were used in a completely randomized design. Current lactation number for control and treatment cows was 3.3 ± 1.3 and 3.0 ± 1.2 , respectively. Previous lactation 305-d milk yield for control and treatment cows was $11,162 \pm 931$ and

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Table 1. Ingredient and nutrient composition of prepartum and lactation TMR¹

Item	Prepartum TMR		Lactation TMR	
Ingredients, % of DM				
Alfalfa silage	11.0		16.7	
Corn silage	48.0		29.6	
Mixed alfalfa-grass hay	—		3.7	
Wheat straw	11.0		—	
Ground shelled corn	18.2		22.1	
Soybean meal-48%	9.2		9.3	
Distillers dried grains	—		9.3	
Whole cottonseed-linted	—		5.6	
Tallow	—		0.9	
Calcium sulfate	0.91		—	
Magnesium sulfate	0.91		—	
Calcium carbonate	—		0.94	
Sodium bicarbonate	—		0.80	
Magnesium oxide	—		0.22	
Mg-K-S ²	—		0.15	
Sodium chloride	—		0.09	
Trace mineral salt ³	0.37		0.37	
Vitamin premix ⁴	0.37		0.19	
Selenium 0.02%	0.04		0.04	
Nutrients ⁵				
	C ⁶	EO ⁶	C	EO
DM, % as fed	47.5 ± 2.1	46.4 ± 3.1	53.2 ± 3.1	53.8 ± 2.7
	DM basis			
CP, %	12.1 ± 0.8	12.1 ± 1.2	17.0 ± 0.4	17.0 ± 0.8
NDF, %	37.0 ± 1.8	37.8 ± 1.5	35.5 ± 2.1	35.0 ± 1.6
Starch, %	27.3 ± 2.2	27.8 ± 2.4	24.3 ± 1.4	25.1 ± 1.5
Fat, %	3.0 ± 0.7	2.8 ± 0.7	5.9 ± 0.2	6.0 ± 0.1
TDN _{1x} , %	68.9 ± 2.0	68.1 ± 2.0	—	—
NE _{L3x} , Mcal/kg	—	—	1.70 ± 0.04	1.72 ± 0.04

¹Treatments were initiated 3 wk before the expected calving date and continued through 15 wk of lactation.

²Dynamate (11% Mg, 18% K, 22% S; The Mosaic Co., Plymouth, MN).

³88% NaCl, 0.002% Co, 0.2% Cu, 0.012% I, 0.18% Fe, 0.8% Mn, 0.006% Se, and 1.4% Zn.

⁴Vitamin A, 3,300,000 IU/kg; vitamin D, 1,100,000 IU/kg; vitamin E, 11,000 IU/kg.

⁵Analysis on TMR samples composited by treatment by month from Dairy One Laboratory (Ithaca, NY); TDN_{1x} (total digestible nutrients) and NE_{L3x} values were calculated according to NRC (2001).

⁶Total mixed rations supplemented with a specific mixture of plant essential oils (Crina, DSM Nutritional Products Inc., Parsippany, NJ) targeted for 1.2 g/cow per day through 62 g/cow per day of premix (EO) or a control (C) carrier premix (62 g/cow per day) without the essential oils mixture.

11,221 ± 1,086 kg, respectively. Disease incidences for control and treated cows, respectively, were retained placenta (2 vs. 1), milk fever (2 vs. 3), ketosis (2 vs. 3), displaced abomasums (2 vs. 2), and mastitis (2 vs. 3). The experimental protocol was approved by the Research Animal and Resource Center of the College of Agriculture and Life Sciences, University of Wisconsin-Madison. Cows received an EO mixture (Crina, DSM Nutritional Products Inc., Parsippany, NJ) targeted for 1.2 g/cow per day of an EO mixture through 62 g/cow per day of premix or a control (C) carrier premix (62 g/cow per day) without EO. The Crina EO was a defined and patented blend of natural and natural-identical EO compounds that included thymol, eugenol, vanillin, and limonene on an organic carrier (McIntosh et al., 2003). Approximately equal proportions of wheat red dog and calcium carbonate either with or without EO comprised the premixes (Vita Plus Corporation,

Madison, WI). The premixes were sampled weekly and composited by month, and the monthly composites were analyzed (DSM Nutritional Products Inc.) for EO concentration. The analyzed EO concentrations in the treatment premix were 1.66% ± 0.12 vs. the formulated target concentration of 1.94% with no relationship ($P = 0.45$ for slope; $R^2 = 0.06$) to month of sampling. Thus, cows received 1.03 ± 0.07 g/d of EO through the premix rather than 1.2 g/cow per day.

Cows were individually fed a TMR that included either the C or EO premix once daily in tie stalls for 10% refusal. The TMR amounts fed and refused were recorded daily. Cows were started on trial 4 wk before expected calving date and continued for 15 wk after calving. During the first week of the trial, cows were fed the prepartum TMR absent of either premix to provide a stall and diet adjustment period. Thus, treatments were initiated 3 wk before calving. The ingredient

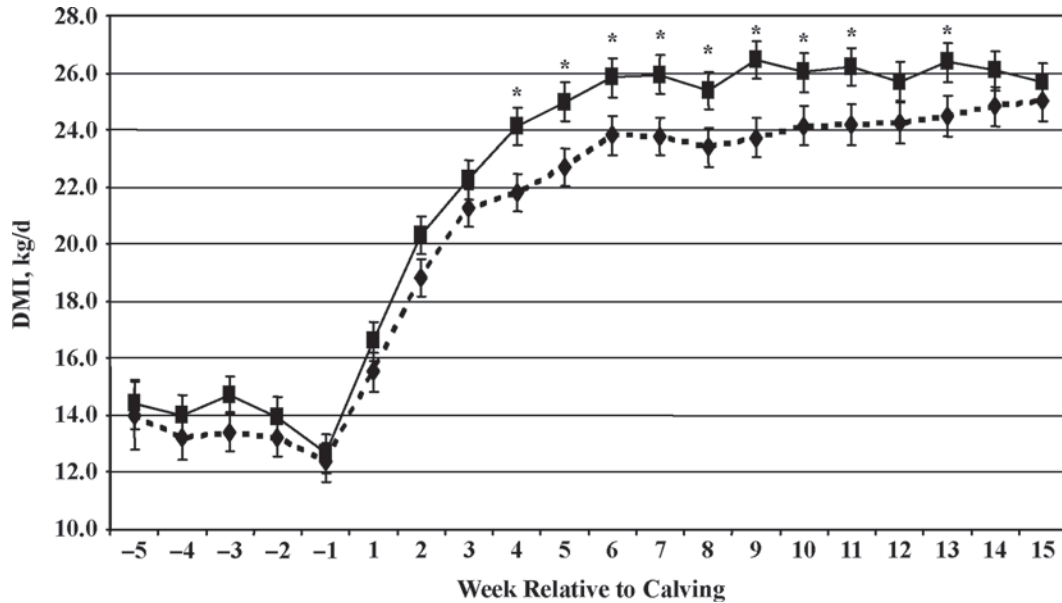


Figure 1. Weekly DMI least squares means for cows fed control (■) and essential oil (◆)-supplemented TMR. * $P < 0.05$.

composition of prepartum and lactating cow TMR are presented in Table 1. The prepartum TMR contained 70% forage comprised of 70% corn silage, 15% alfalfa silage, and 15% wheat straw (DM basis). The lactation TMR contained 50% forage comprised of 60%

corn silage, 33% alfalfa silage, and 7% alfalfa hay (DM basis). Prepartum and lactation TMR were formulated to contain 12 and 17% CP (DM basis), respectively, and to meet or exceed NRC (2001) mineral and vitamin guidelines. All cows were injected with bST (Posilac,

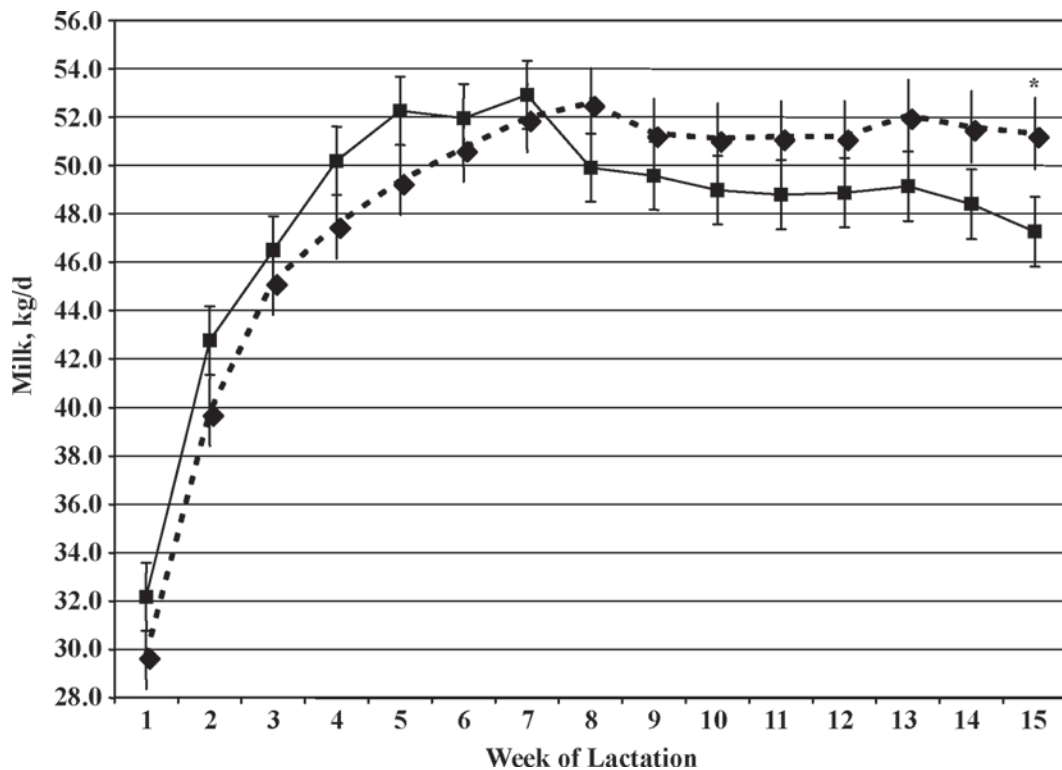


Figure 2. Weekly milk yield least squares means for cows fed control (■) and essential oil (◆)-supplemented TMR. * $P < 0.10$.

Table 2. Effect of supplemental dietary essential oils on least squares means for DMI and performance of periparturient and early lactation cows¹

Item	C	EO	SEM	<i>P</i> <
DMI				
kg/d				
Prepartum ²	13.8	13.1	0.4	NS ³
Lactation ²	24.5	22.7	0.6	0.04
% of BW				
Prepartum	1.85	1.77	0.11	NS
Lactation	3.67	3.47	0.07	0.07
Milk yield, kg/d	48.2	48.1	1.1	NS
4% FCM, kg/d	43.9	44.0	1.2	NS
Milk fat				
%	3.48	3.46	0.10	NS
kg/d	1.65	1.64	0.09	NS
Milk protein				
%	3.10	2.95	0.05	0.03
kg/d	1.46	1.41	0.06	NS
MUN, mg/dL	12.9	13.4	0.3	NS
Feed efficiency				
kg of milk/kg of DMI	1.99	2.15	0.06	0.08
kg of FCM/kg of DMI	1.83	1.98	0.06	0.07
BCS				
Prepartum	3.9	3.8	0.1	NS
Lactation	3.4	3.3	0.1	NS
BW, kg				
Prepartum	734	745	16	NS
Lactation	672	658	16	NS
EB, Mcal/d ⁴	-1.1	-3.6	0.9	0.06

¹Total mixed rations supplemented with a specific mixture of plant essential oils (Crina, DSM Nutritional Products Inc., Parsippany, NJ) targeted for 1.2 g/cow per day through 62 g/cow per day of premix (EO) or a control (C) carrier premix (62 g/cow per day) without the essential oils mixture.

²Treatments were initiated 3 wk before the expected calving date and continued through 15 wk of lactation.

³Not significant (*P* > 0.10).

⁴Energy balance (EB) = (DMI × diet NE_L) - [(0.08 × BW^{0.75}) + (milk yield × NE_L)] (NRC, 2001).

Monsanto Company, St. Louis, MO) every 14 d starting in wk 10 of lactation.

Body weights and condition scores (Wildman et al., 1982) were recorded weekly on all cows throughout the trial at the same time on the same day each week. Individual cow milk yields were recorded daily. Milk samples were collected from all cows on 2 consecutive milkings on the same day each week and analyzed for fat, true protein, lactose, and MUN concentrations using infrared analysis (AgSource Milk Analysis Laboratory, Menomonie, WI). Fat-corrected milk (4%) and milk NE_L (Mcal/kg) were calculated using equations from NRC (2001). Energy balance (**EB**) was calculated using the following equation (NRC, 2001):

$$\text{EB} = (\text{DMI} \times \text{diet NE}_L) - [(0.08 \times \text{BW}^{0.75}) + (\text{milk NE}_L \times \text{milk yield})].$$

Weekly TMR samples were collected and composited by treatment by month at Dairy One Laboratory (Ithaca, NY) using wet chemistry analysis (Dairy One, 2007) for DM, CP, NDF, NDF-CP, fat, ash, and starch

with NFC and energy values calculated from the analytical data using NRC (2001) equations.

Blood samples were collected from the coccygeal vein before feeding -21, -14, and -7 d relative to expected calving. Samples for d -1 were taken every other day from d -1 relative to expected calving until the cow calved to obtain an analysis as close to d -1 as possible. Postpartum blood samples were taken 1, 8, 15, 22, and 29 d relative to calving. Samples were collected in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing either potassium oxalate and sodium fluoride or ethylene diamine tetraacetic acid and were centrifuged for 15 min at no less than 625 × *g*. Plasma was aliquoted into 1-mL tubes and tubes were frozen until analyzed for NEFA (NEFA-C kit, Wako Chemical USA, Richmond, VA; Johnson and Peters, 1993), glucose (glucose oxidase-peroxidase method; Karkalas, 1985), BHBA (Gibbard and Watkins, 1968), and plasma urea-N (Chaney and Marback, 1962).

Data were analyzed as a completely randomized design using PROC MIXED of SAS (SAS Institute, 2004) with time as repeated measures using the first-order autoregressive covariance structure, which provided the

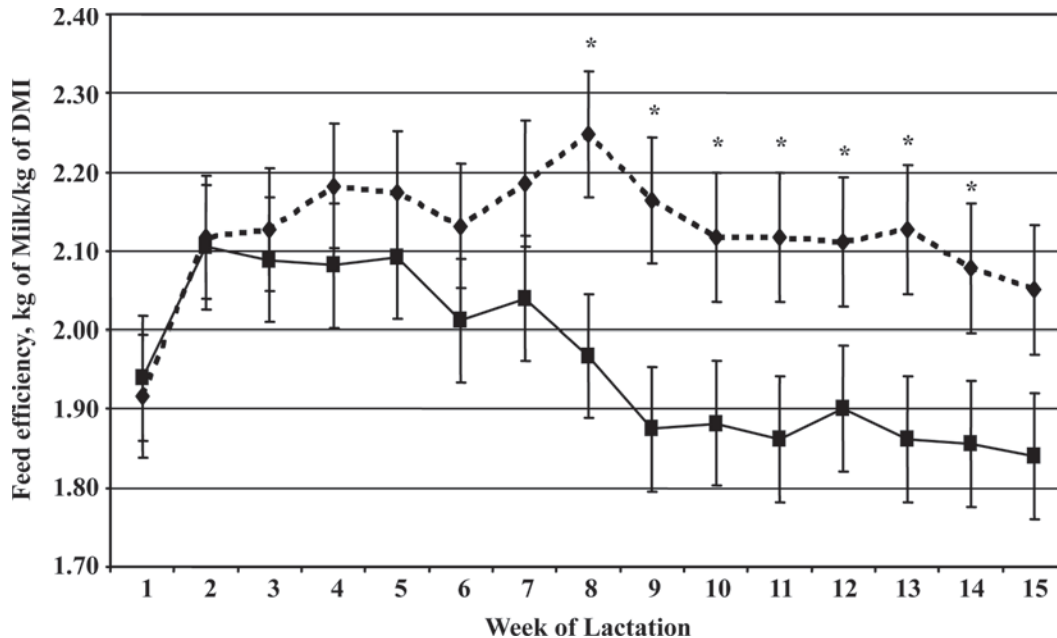


Figure 3. Weekly feed efficiency (kg of milk/kg of DMI) least squares means for cows fed control (■) and essential oil (◆)-supplemented TMR. * $P < 0.05$.

best fit according to Sawa's Bayesian information criterion. The model included treatment, time (week for periparturient and early lactation measurements and day for blood measurements), and treatment \times time interaction as fixed effects and cow within treatment as a random effect. Degrees of freedom were calculated using the Kenward-Roger option. Means were determined using the least squares means statement. Statistical significance and trends were considered at $P \leq 0.05$ and $P \geq 0.06$ to $P < 0.10$, respectively.

RESULTS AND DISCUSSION

The nutrient composition of prepartum and lactation TMR for C and EO are presented in Table 1. Prepartum and lactation TMR nutrient composition was similar to the diet formulation targets. The TMR for C and EO treatments were of similar nutrient composition.

Presented in Table 2 are results for DMI and performance of periparturient and early lactation cows. Dry matter intake of prepartum cows was unaffected by treatment and lactation DMI was less ($P < 0.05$) for EO (22.7 kg/d) than C (24.5 kg/d). This response is in contrast to other research that showed either increased DMI (Kung et al., 2008) or no difference ($P > 0.10$) in DMI (Benchaar et al., 2006, 2007) for CRINA EO-supplemented versus nonsupplemented dairy cows. Cows in those trials were in a later stage of lactation than cows in our study. The DMI by week data for our trial are presented in Figure 1, which shows that

C cows ate more ($P < 0.05$) than EO cows during wk 4 to 11 and 13. A possible explanation for the decreased DMI with EO supplementation could be that EO adversely influenced the palatability of the TMR fed in this study. This premise seems unlikely, though, because DMI was unaffected by treatment during the prepartum period and wk 1 to 3 of lactation. We made no feeding behavior measurements in our study to be able to assess palatability differences between the control and treatment TMR. To our knowledge, this is the first report of intake responses to EO supplementation in periparturient cows, and more research is needed.

Milk yield averaged 48 kg/d and was unaffected by treatment in agreement with the reports of Benchaar et al. (2006, 2007). Kung et al. (2008), however, reported that dietary supplementation with CRINA EO increased milk yield. Average daily milk yields for C and EO summarized by week are presented in Figure 2. Milk yield tended ($P < 0.10$) to be higher for EO at wk 15. Continuing our trial further into lactation may have revealed a continuation of this trend, but we have no direct data to support this premise. Kung et al. (2008), however, reported a milk yield increase from CRINA EO supplementation in midlactation cows. It is unclear why milk yield responses to EO would differ depending on stage of lactation, and this question needs further study. Milk protein percentage was reduced ($P < 0.05$) for EO (2.95 vs. 3.10%).

We observed trends ($P < 0.10$) for increased average milk (2.15 vs. 1.99 kg/kg of DMI) and FCM feed

Table 3. Effect of supplemental dietary essential oils on least squares means for plasma concentrations of glucose, NEFA, BHBA, and urea-N in periparturient and early lactation cows¹

Parameter	C	EO	SEM	<i>P</i> < ²
Glucose, mg/dL				
Prepartum ³	56.6	57.8	1.0	NS
Lactation ⁴	52.1	53.3	0.9	NS
NEFA, mEq/L				
Prepartum	280	299	35	NS
Lactation	671	670	34	NS
BHBA, mg/dL				
Prepartum	4.8	5.5	0.6	NS
Lactation	7.9	9.2	0.6	NS
Urea-N, mg/dL				
Prepartum	10.4	10.1	0.3	NS
Lactation	12.9	13.1	0.3	NS

¹Total mixed rations supplemented with a specific mixture of plant essential oils (Crina, DSM Nutritional Products Inc., Parsippany, NJ) targeted for 1.2 g/cow per day through 62 g/cow per day of premix (EO) or a control (C) carrier premix (62 g/cow per day) without the essential oils mixture. Treatments were initiated 3 wk before the expected calving date and continued through 15 wk of lactation.

²Treatment effects were not significant (*P* > 0.10). Time effects (*P* < 0.05) were observed for all parameters measured. No interactions of treatment × time were detected (*P* > 0.10).

³Blood samples collected −21, −14, −7, and −1 d relative to calving.

⁴Blood samples collected at 1, 8, 15, 22, and 29 DIM.

efficiencies (1.98 vs. 1.83 kg/kg of DMI) and decreased average EB (−3.6 vs. −1.1 Mcal/d) for EO (Table 2). Body weight and BCS measurements were unaffected by treatment. The change in BCS between calving and wk 15 was 1.1 units for both C and EO. Feed efficiency responses by week are presented in Figure 3; differences (*P* < 0.05) were observed during wk 8 to 14. Monensin is a rumen antimicrobial that has been shown to reduce DMI and increase feed efficiency in dairy cows (Ipharaguerre and Clark, 2003). Benchaar et al. (2006) evaluated monensin (350 mg/d) and EO (CRINA; 750 mg/d) in lactating dairy cows and reported an interaction (*P* < 0.04) between the treatments for DMI (% of BW); EO increased DMI when supplemented with monensin but decreased DMI when supplemented alone.

Results for plasma concentrations of glucose, NEFA, BHBA, and urea-N in periparturient and early lactation cows are presented in Table 3. Although time effects (*P* < 0.05) were observed for all parameters measured, no interactions of treatment × time were detected (*P* > 0.10). Therefore, data averaged separately across the prepartum and early lactation sampling time points are presented in Table 3 and no figures are provided for the blood parameters. Blood measurements were unaffected by treatment. With the observed trend for a more negative EB in EO cows, increases in plasma NEFA and BHBA concentrations may have been expected (Grummer, 1993). However, DMI was unaffected by treatment during the prepartum period and wk 1 to 2 of lactation and EB was unaffected by treatment dur-

ing wk 1 to 3 of lactation. The periparturient period is the critical time influencing plasma NEFA and BHBA concentrations (Grummer, 1993) and because most of our blood samples were obtained before the onset of differences between the treatments for EB, this could explain our lack of treatment effects for these blood parameters. It is important to note again that BW and BCS measurements were unaffected by treatment. Considering the combined results for DMI, BW, and blood measurements, there was no influence of EO in prepartum cows.

CONCLUSIONS

There was no benefit to the dietary supplementation of EO for prepartum cows. The dietary supplementation of EO in early lactation cows decreased DMI 1.8 kg/d on average, whereas milk yield was maintained similar to the control at 48 kg/d.

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