

# Effects of Specific Essential Oil Compounds on, Feed Intake, Milk Production, and Rumenal Environment in Dairy Cows during Heat Exposure

K. Reza-Yazdi, M. Fallah, M. Khodaparast, F. Kateb, M. Hosseini-Ghaffari

**Abstract**—The objective of this study was to determine effect of dietary essential oil (EO) compounds, which contained cinnamaldehyde, eugenol, peppermint, coriander, cumin, lemongrass, and an organic carrier on feed intake, milk composition, and rumen fermentation of dairy cows during heat exposure. Thirty-two Holstein cows (days in milk =  $60 \pm 5$ ) were assigned to one of two treatment groups: a Control and EO fed. The experiment lasted 28 days. Dry matter intake (DMI) was measured daily while and milk production was measured weekly. Our result showed that DMI and milk yield was decreased ( $P < 0.01$ ) in control cows relative to EO cows. Furthermore, supplementation with EO was associated with a decrease in the molar proportion of propionate ( $P < 0.05$ ) and increase ( $P < 0.05$ ) in acetate to propionate ratio. In conclusion, EO supplementations in diets can be useful nutritional modification to alleviate for the decrease DMI and milk production during heat exposure in lactating dairy cows.

**Keywords**—Dairy cow, feed additive, plant extract.

## I. INTRODUCTION

ESSENTIAL OILS (EO) are the volatile and aromatic compounds that can be as alternatives to feed antibiotics in animal production [1]. Several investigations have studied the influence of various EO on production performance of dairy cows [2]-[4]. Numerous results show that EO have bioactivities, such as selective anti-bacterial activity, inhibition of ruminal methane emission, as well as enhancement of ruminal propionate proportion and bypass protein to the intestine [5], [8]. In the *in vivo* study, feeding a blend of thymol, eugenol, vanillin, guaiacol, and limonene at a moderate dose (600 mg/d) increased DMI and 4% fat corrected milk (FCM) of lactating dairy cows, but it did not affect intake or production at greater doses (750 mg/d) of dairy cows [5]. However, feeding a high dose (1.2 g/d) of that same blend of EO (included thymol, eugenol, vanillin, and limonene on an organic carrier) had no effect on dairy cows milk production [6]. Previous study [2] reported that feeding

EO complex (1 g/cow/d; contained eugenol, geranyl acetate and coriander oil) impacted the production of high producing dairy cows, primarily by enhancing milk fat synthesis which apparently was the result of an energetic shift away from body condition gain, suggesting that the EO complex may have enhanced acetate production and/or the ratio of acetate to propionate production in the rumen. Reference [7] reported that a blend of eugenol, and cinnamaldehyde fed at moderate doses (~500 mg/d) increased both the fat and protein contents of milk, but had no effect on DMI or milk production. Reference [8] noted that plant extracts cinnamaldehyde, eugenol, and capsicum have antimicrobial properties and may modify the rumen environment. The positive effects of EO, including increased propionate and decreased ammonia nitrogen and methane production without reducing total VFA production, have been observed in a review study [8].

In recent years, more research has been directed toward evaluating the potential of EO to improve performance in dairy cows, but to date a few research has focused on the use of EOs in diets fed to heat-stressed dairy cows. Heat stress negatively impacts production of lactating dairy cows [9]. Reference [10] indicate that inclusion of plant extracts (a blend of yeast, *Aspergillus niger*, capsicum, cinnamaldehyde, and eugenol) in diets fed to lactating dairy cows experiencing heat stress did not alter milk yield, but did affect milk components. Many aromatic herbs and essential oils are used for improving the flavour and palatability of animal feed [11]. BioHerbal<sup>®</sup> is a flavor enhancer derived from essential oil compounds with claims such as, appetite stimulant, and digestion stimulant. To our knowledge no studies have so far investigated the impact of the dietary supplementation of EO (BioHerbal<sup>®</sup>) additive on intake, and milk yield dairy cows during heat stress. The present study thus aimed to investigate the effects of an EO blend additive on intake, rumen fermentation parameters, milk yield and composition in dairy cows, and to elucidate whether or not the EO diet supplementation may be used as a protective stress compound.

## II. MATERIAL AND METHODS

### A. Experimental Location and Animal Care

The experiment was conducted at was conducted at the Natural Resources & Agricultural research farm of the Tehran University Karaj, Iran according to the guidelines of the Iranian Council of Animal Care [12].

K. Reza-Yazdi is with the Department of Animal Science, University College of Agriculture and Natural Resources, University of Tehran, Karaj, P.O. Box 3158711167-4111, Iran (e-mail: rezayazdi@ut.ac.ir).

M. Fallah and M. Khodaparast are with the Powerin, Co., Tehran, Iran (e-mail: m.fallah118@gmail.com, mehdi\_khodaparast@yahoo.com).

F. Kateb is with the Behboddan Parsian, Co., Isfahan, Iran (e-mail: dr.kateb@yahoo.com).

M. Hosseini-Ghaffari is with the Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan 84156 – 83111, Iran (Corresponding author: +98 935 781 4911; fax: +983113913471; e-mail: morteza.h.g@gmail.com).

### B. Animals and Treatments

A specific blend (BioHerbal®) of EO compounds manufactured by Pars-Imen-Daru Herbal Medicines Development Co., (Tehran, Iran) was used in this experiment. BioHerbal® was a defined and patented blend of natural and natural-identical EO compounds that included cinnamaldehyde, eugenol, peppermint, coriander, cumin, lemongrass, and an organic carrier. Thirty-two multiparous Holstein cows were randomly assigned to either control or EO-supplemented (2 g/cow per day) total mixed rations (TMR). At the beginning of the trial cows were  $60 \pm 5$  DIM with an average daily milk production of  $36.2 \pm 1.2$  kg/d, and an average BW of  $650 \pm 16$  kg. The TMR contained 40% forage and 60% concentrate; DM basis. The experimental period lasted for 4 weeks.

Cows were individually fed a TMR that included either the control or EO premix once daily in tie stalls for *ad libitum* intake (5 to 10% refusals). BioHerbal® was premixed with a small amount (approximately 100 g) of the corn grain and then top-dressed and mixed with a portion of the TMR before feeding.

### C. Sampling and Measurements

The TMR amounts fed and refused were recorded daily. Animals were milked three times a day at 0600, 1400 and 2200 h and individual milk weights were measured at each milking. Milk samples were pooled to the corresponding milk yield and kept at room temperature (i.e., 23°C) with the preservative potassium dichromate. Milk samples were analyzed for fat, protein, lactose, and non-fat-solids by Milk-O-Scan (134 BN, Foss Electric, Hillerød, Denmark). At the end of experiment, the rumen fluid samples were collected by stomach tube 4 h after morning feeding and strained through four layers of cheesecloth to obtain rumen fluid. Immediately, rumen pH was measured using a handheld pH meter (HI 8314 membrane pH meter, Hanna Instruments, Villafranca, Italy). Four mL of rumen fluid was acidified with 1 mL of 25% metaphosphoric acid and stored (-20°C) until analysis for VFA. Rumen samples were analyzed for VFAs by gas chromatography (model CP-9002, Chrompack, Middelburg, The Netherlands) with a 50 m (0.32 mm ID) silica-fused column (CP-Wax Chrompack Capillary Column, Varian, Palo Alto, CA, USA). Helium was used as carrier gas and oven initial and final temperatures were 55 and 195°C, respectively. Detector and injector temperatures were set at 250°C. Crotonic acid (1:7, v/v) was used as the internal standard.

### D. Statistical Analyses

All data from were analyzed using the PROC MIXED procedure of SAS software (SAS Institute, Inc.; Version 9.1) [13], with the animal as the experimental unit according to the following model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where;  $y_{ij}$  = dependent variable;  $\mu$  = overall mean of the population;  $T_i$  = treatment, and  $\varepsilon_{ij}$  = unexplained residual element assumed to be independent and normally distributed.

When differences ( $P < 0.05$ ) due to interactions or dietary treatments were detected, means separation was conducted using a Tukey adjustment for the probability. Significance was declared at  $P \leq 0.05$  and trends were considered at  $0.05 < P < 0.10$ .

## III. RESULTS AND DISCUSSION

The effects of supplementation of dairy cows with EO (2 g/d) on DMI, milk production and composition variables are presented in Table I. Dry matter intake was decreased ( $P < 0.01$ ) in control cows relative to EO cows. The DMI by week data for our study are presented in Fig. 1, which shows that control cows fed less ( $P < 0.01$ ) DMI compared with EO cows during week 3 to 4 under the heat stress. In agreement with our findings, [3] conclude that a blend of eugenol (28%) and cinnamaldehyde (17%) can increase DMI and milk production in lactating dairy cows, and environmental factors appear to influence the response to EO, including dose and parity. A possible explanation for no reduction in DMI with EO supplementation could be that EO positively influenced the palatability of the TMR fed in this study. In contrast with our results, [6] noted that the dietary supplementation of EO (1.2 g/cow per day) 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. Reference [10] also indicate that inclusion of plant extracts a blend of *Pichia guilliermondii*, *Aspergillus niger*, and capsicum in diets fed to lactating dairy cows experiencing heat stress did not alter DMI or milk yield. Reference [15] reported that the blend of EO (Crina, DSM Nutritional Products Ltd., 40% active ingredients) reduced the molar proportion of acetate but increased the molar proportion of propionate, which may partially explain why, when fed to lactating dairy cows; the blend of EO increased DMI and 3.5% FCM production. Reference [14] summarized that feeding a mixture of natural and synthesized EO, including thymol, eugenol, vanillin, guaiacol, and limonene, may increase DMI and feed efficiency, milk yield and composition (fat and protein percentages of dairy cows).

TABLE I  
EFFECT OF SUPPLEMENTAL DIETARY ESSENTIAL OILS ON LEAST SQUARES MEANS FOR DMI, MILK PRODUCTION AND COMPOSITION OF DAIRY COWS<sup>1</sup>

Item	Control	EO mixture	SEM	P-value
DMI	22.8 <sup>b</sup>	24.7 <sup>a</sup>	0.14	**
Milk production (kg/d)	34.9 <sup>b</sup>	37.4 <sup>a</sup>	0.55	**
Milk Composition (%)				
Fat	3.48	3.53	0.03	NS
Protein	3.19	3.18	0.02	NS
Lactose	4.71	4.76	0.03	0.06

<sup>1</sup> Total mixed rations supplemented with a specific mixture of plant essential oils (BioHerbal®, Pars-Imen-Daru Herbal Medicines Development Co., Tehran, Iran) targeted for 2 g/cow per day of premix (EO) or a control without the essential oils mixture.

\*\* = significant ( $P < 0.01$ ). NS = Not significant ( $P > 0.05$ ).

However, our results is in contrast to other findings that have shown either increased DMI [15] or no difference ( $P > 0.05$ ) in DMI [5] for EO-supplemented versus control cows. Various responses to EO supplements on DMI of dairy cows

may be attributed to differences in dose, duration and processing of the medicinal plants, EO and/or to the different husbandry conditions [16]. It is unclear how EO modulates feed intake, but the effect might be partly attributed to the observed apparent improvement in rumen function, increased fiber digestibility [4].

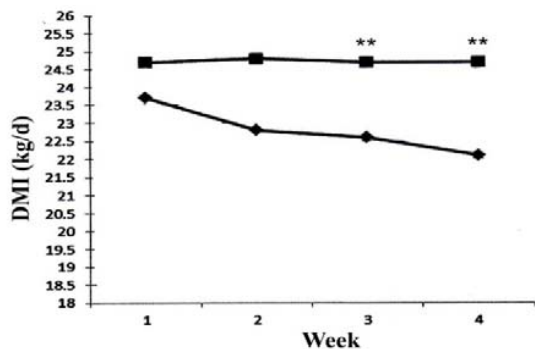


Fig. 1 Weekly DMI least squares means for cows fed control (◆) and essential oil (■)-supplemented TMR. \*\*  $P < 0.01$

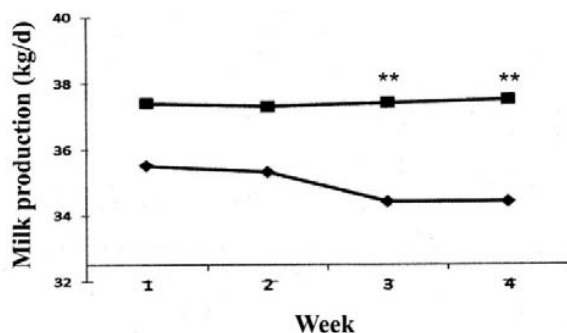


Fig. 2 Weekly milk production least squares means for cows fed control (◆) and essential oil (■)-supplemented TMR. \*\*  $P < 0.01$ .

In dairy cows, milk production was decreased in control cows compared with EO cows ( $P < 0.01$ ; Fig 2). We observed no effect ( $P > 0.05$ ) of EO on milk fat, and protein, although we identified a trend ( $P = 0.06$ ) for an increase in lactose in EO cows. Reference [15] reported that dietary supplementation with EO (Crina, DSM Nutritional Products Ltd., 40% active ingredients) increased milk yield and 3.5% FCM in mid-lactation cows, which is in agreement with our findings.

The mean of ruminal fermentation parameters is presented in Table II. We observed no effect ( $P > 0.05$ ) of EO on the concentration of total VFA, and the molar proportion of acetate and butyrate. In the current study, supplementation with EO was associated with a decrease in the molar proportion of propionate ( $P < 0.05$ ) and increase ( $P < 0.05$ ) in acetate to propionate ratio. The lack of effect of EO was attributed to a possible adaptation of rumen microbes to these compounds. Several studies have been reported that total VFA concentrations could increase [16], decrease [17], or be unaffected [18], [5], [19] when different EO compounds are supplemented to ruminant diets. Reference [14] summarized

that feeding a mixture of natural and synthesized EO, including thymol, eugenol, vanillin, guaiacol, and limonene, increased ruminal pH and reduce total VFA in dairy cows. Clearly, many of these studies confirm the potential effects of EO to influence lactation performance of dairy cows; however, they also confirm the observations of previous *in vitro* experiments that the source and dose of the EO used can have marked effects on the response of the animal. Reference [3] reported that EO fed at 525 mg/d to lactating dairy cows had only moderate effects on rumen fermentation and decreased the acetate: propionate ratio with no effect on rumen protozoa. Reference [5] showed that when used at more normal feeding doses, EO (750 mg/d; Crina ruminants; CRINA S.A., Gland, Switzerland) had no effect on rumen microbial fermentation, digestion, and dairy cow performance. Supplementation of ruminant diets with EO can alter microbial populations, and rumen fermentation of diets in the rumen [8]. Reference [20] indicated that garlic oil, cinnamaldehyde, eugenol, capsaicin and anethol improve the fermentation profile *in vitro* study.

TABLE II  
EFFECT OF SUPPLEMENTAL DIETARY ESSENTIAL OILS ON LEAST SQUARES MEANS FOR RUMEN FERMENTATION PARAMETERS OF DAIRY COWS<sup>1</sup>

Item	Control	EO mixture	SEM	P-value
Ruminal pH	6.05 <sup>b</sup>	6.33 <sup>a</sup>	0.03	**
Total VFA (mmol/l)	125.5	116.2	6.97	NS
Individual VFA (mmol/100 mol)				
Acetate	88.9	84.6	5.05	NS
Propionate	22.0 <sup>a</sup>	18.2 <sup>b</sup>	1.37	*
Butyrate	12.0	11.3	0.79	NS
C2:C3 <sup>2</sup>	4.1 <sup>b</sup>	4.7 <sup>a</sup>	0.18	*

<sup>1</sup> Total mixed rations supplemented with a specific mixture of plant essential oils (BioHerbal®, Pars-Imen-Daru Herbal Medicines Development Co., Tehran, Iran) targeted for 2 g/cow per day of premix (EO) or a control without the essential oils mixture.

<sup>2</sup> Acetate to propionate ratio.

\* = significant ( $P < 0.05$ ). \*\* = significant ( $P < 0.01$ ). NS = Not significant ( $P > 0.05$ ).

#### IV. CONCLUSION

Addition of a specific mixture of EO compounds (BioHerbal®) had significant effects on milk production, ruminal pH, and the acetate to propionate ratio in the rumen. We conclude that supplementation with a blend of EO can be useful nutritional modification to alleviate for the decrease DMI and milk production in lactating dairy cows during the heat exposure. Further investigations are required to quantify the physiological effects of EO dairy during heat stress.

#### ACKNOWLEDGMENT

This study was financed by Pars-Imen-Daru Herbal Medicines Development Co., (Tehran, Iran). The authors appreciate the employees of the Natural Resources & Agricultural research farm of the Tehran University Karaj for animal care. We would also like to thank Dr. Saeed Rasoulnezhad for his insightful comments on this paper.

## REFERENCES

- [1] R. J. Wallace, "Antimicrobial properties of plant secondary metabolites," *Proceedings of the Nutrition Society*, vol. 63, pp. 621–629, 2004.
- [2] M. B. Santos, P. H. Robinson, P. Williams, and R. Losa, "Effects of addition of an essential oil complex to the diet of lactating dairy cows on whole tract digestion of nutrients and productive performance," *Animal Feed Science and Technology* vol. 157 pp. 64–71. 2010.
- [3] J. A. Tekippe, R. Tacoma, A. N. Hristov, C. Lee, J. Oh, K. S. Heyler, T. W. Cassidy, G. A. Varga, and D. Bravo, "Effect of essential oils on ruminal fermentation and lactation performance of dairy cows," *Journal of Dairy science*, vol. 96 pp. 7892–7903, 2013.
- [4] E. H. Wall, P. H. Doane, S. S. Donkin, D. Bravo, "The effects of supplementation with a blend of cinnamaldehyde and eugenol on feed intake and milk production of dairy cows," *Journal of Dairy science*, vol. 97 pp. 25709-5717. 2014.
- [5] C. Benchaar, H. V. Petit, R. Berthiaume, D. R. Ouellet, J. Chiquette, and P. Y. Chouinard, "Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage," *Journal of Dairy science* vol. 90 pp. 886–897, 2007.
- [6] M. D. Tassoul, and R. D. Shaver, "Effect of a mixture of supplemental dietary plant essential oils on performance of periparturient and early lactation dairy cows," *Journal of Dairy science* vol. 92 pp. 1734–1740, 2009.
- [7] M. A. Spanghero, Z. M. Salem, and P. H. Robinson, "Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces," *Animal Feed Science and Technology* vol. 152 pp. 243–255. 2009.
- [8] S. Calsamiglia, M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret. Invited review: Essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy science* vol. 90 pp. 2580–2595, 2007.
- [9] R.B., Zimbelman, Collier, R.J., Bilby, T.R. , "Effects of utilizing rumen protected niacin on core body temperature as well as milk production and composition in lactating dairy cows during heat stress," *Animal Feed Science and Technology*, vol. 180 pp. 26-33, 2013.
- [10] J. Boyd, J. W. West, J. K. Bernard, and S. S. Block, "Effects of plant extracts on milk yield and apparent efficiency of lactating dairy cows during hot weather," *Professional Animal Science* vol. 28 pp. 338–343, 2012.
- [11] C., Franz, K. H. C. Baser and W. Windisch "Essential oils and aromatic plants in animal feeding - a European perspective. A review," *Flavour and Fragrance Journal* vol. 25 pp. 327-340, 2010.
- [12] Iranian Council of Animal Care, "Guide to the Care and Use of Experimental Animals, Vol. 1. *Isfahan University of Technology, Isfahan, Iran*, 1995.
- [13] SAS Institute. SAS User's Guide: Statistics. *Release 9.1. SAS Institute Inc., Cary, NC*. 2002.
- [14] R. D. Shaver, M. D. Tassoul, "Essential oils as dietary supplements for dairy cows. *Mid-Atlantic Nutrition Conference, Baltimore, MD*," 2008.
- [15] J. L. Kung, P. Williams, R. J. Schmidt, and W. Hu, "A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows," *Journal of Dairy science* vol. 91 pp. 4793–4800, 2008.
- [16] L. Castillejos, S. Calsamiglia, A. Ferret, and R. Losa, "Effects of a specific blend of essential oil compounds and the type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system," *Animal Feed Science and Technology* vol. 119 pp. 29-41, 2005.
- [17] G. Varga, E. Block, P. Williams, T. W. Cassidy, and R. Losa, "Effect of CRINA ruminants, a mixture of essential oil components, on continuous culture fermentation and milk production of lactating cows. *Journal of Animal Science* vol. 82 pp. 334. 2004.
- [18] C. J. Newbold, F. M. McIntosh, P. Williams, R. Losa, and R. J. Wallace, "Effects of a specific blend of essential oil compounds on rumen fermentation," *Animal Feed Science and Technology* vol. 114 pp. 105–112. 2004.
- [19] L. Castillejos, S. Calsamiglia, A. Ferret, and R. Losa, "Effects of dose and adaptation time of a specific blend of essential oil compounds on rumen fermentation. *Animal Feed Science and Technology* vol. 132 pp. 186-201, 2007.
- [20] P. W. Cardozo, S. Calsamiglia, A. Ferret, and C. Kamel, "Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture," *Journal of Animal Science* vol. 82 pp. 3230–3236. 2004.