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Effect of Essential Oils of Various Plants as Microbial Modifier to Alter Corn Silage Fermentation and *in vitro* Methane Production

Research Article

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ABSTRACT

The aim of present study was to assess the effects of individual various essential plant oils on chemical composition, aerobic stability and fermentation characteristics of corn silage and *in vitro* rumen fermentation and methane emission using gas production technique. Whole crop corn was harvested and ensiled in laboratory scale tubes (4±0.25 kg) for 45 days. Treatments consisted of either corn silage with no additive (control) or treated with 120 or 240 mg kg^{-1} DM of cinnamon (Cinn120 or Cinn240, respectively), thyme (Thy120 or Thy240, respectively), mint (Mint120 or Mint240, respectively), oregano (Oreg120 or Oreg240, respectively) or cumin (Cum120 or Cum240, respectively). Treatments were compared with control using the Dunnett test at ($P<0.05$). Silages treated with cinnamon essential oil had higher DM content compared with the control ($P<0.05$). The pH value of Mint240 was significantly lower than the control ($P<0.05$). A significant reduction in ammonia nitrogen concentration of silages treated with thyme essential oil was observed in comparison to the control ($P<0.05$). Relative to the control, all of applied additives except of mint essential oil caused a significant improvement in silage aerobic stability (ranging from 44% to 400% increment). At 24 h *in vitro* rumen post incubation, total produced gas from Oreg240 was diminished significantly ($P<0.05$) compared with the control (44.23 *vs.* 48.73 mL 250 mg^{-1} DM). When compared to the control, all treated silages except of Mint240 or Oreg120 produced higher methane during 24 h incubation ($P<0.05$). However, the ratio of methane to total gas was not affected by experimental treatments ($P>0.05$). *In vitro* dry matter disappearance of Thy120, Mint240 or Oreg240 increased by 10 percent compared with the control ($P<0.05$). Moreover, partitioning factor of Oreg240 was higher than the control ($P<0.05$). Results demonstrated the potential of applied essential oils to improve chemical composition, dry matter degradability and aerobic stability of corn silage.

KEY WORDS essential oil, methane emission, silage fermentation.

INTRODUCTION

During ensiling process, microbial populations present in forage mass consume forage carbohydrates as their energy source and produce different metabolite such as lactic acid, acetic acid and butyric acid. Lactic acid production by *Lactobacillus* species decreases the pH of silage (Kung and Ranjit, 2001) which could restrict activity of undesirable microorganism such as *Clostridia* (Duniere *et al.* 2011). *Clostridia* convert forage carbohydrates to butyric acid which could lead to increase in pH and further spoilage of the silage (Dunière *et al.* 2013). In order to ensure *Lactobacillus* predominating fermentation and high concentration of lactic acid in silage, various additives were tested (Dunie`re *et al.* 2013; Yitbarek and Tamir, 2014). Essential oils are plant secondary metabolites (PSM) which are re-

sponsible for the odor and color of them (Balandrin and Klocke, 1985) and commonly extracted from different plant tissues by distillation methods (Losa, 2001). Many studies reported the stimulatory and inhibitory effects of essential oils on vast variety of microorganisms under *in vitro* and *in vivo* conditions (Newbold *et al.* 2004; Benchaar *et al.* 2007; Nanon *et al.* 2014; Khorrami *et al.* 2015). For example, Fraser *et al.* (2007) evaluated the effects of cinnamon essential oil on rumen microbial fermentation using continuous culture system and observed a reduction in ammonia nitrogen (NH_3-N) concentration of cultural medium. McIntosh *et al.* (2003) also observed inhibition of hyper ammonia-producing bacteria by a commercial blend of essential oils. Moreover, some of former studies demonstrated the potential of these compounds to alter rumen fermentation by reducing the proportion of acetate to propionate and also inhibition of methanogenesis (Busquet *et al.* 2005; Benchaar and Greathead, 2011). All of these studies used essential oils directly in ruminant feed or in cultural medium. Based on available information, very limited numbers of studies have assessed the effects of some essential oils on silage fermentation. Kung *et al.* (2008) supplemented corn silage with different doses of a specific blend of essential oils and observed no improvement in silage fermentation characteristics and in aerobic stability of corn silage. However, in study of Chaves *et al.* (2012), addition of cinnamon leaf, oregano and sweet orange essential oils in barley silage decreased yeast growth during aerobic stability test. The present study was conducted to determine the effects of essential oils of cinnamon, thyme, mint, oregano or cumin on chemical composition, aerobic stability and fermentation characteristics of corn silage and to evaluate the effect of the silages on *in vitro* rumen fermentation pattern and methane production using gas production technique.

MATERIALS AND METHODS

Essential oils preparation

Cinnamon (*C. verum*), thyme (*T. vulgaris*), mint (*M. spicata*), oregano (*O. vulgare*) and cumin (*C. cyminum*) used in this study were purchased from local markets. The plant obtained was cut or crushed into small pieces, oven-dried at 39 ˚C for 48 h and ground to pass a 1 mm-screen. Essential oils content of each plant was obtained with hydro-distillation of grinded samples using Clevenger apparatus (Jahani-Azizabadi *et al.* 2014). The essential oils were stored in refrigerator (4 ˚C) until they were used in the experiment.

Ensiling procedure

Whole crop corn was sampled every other day to measure dry matter (DM) content by air forced oven (65 ˚C for 48 h) and harvested when reached at 29%. Then it was chopped at 20 mm length and ensiled in laboratory scale polyvinyl tubes (4±0.25 kg) for 45 days. The forage treated with no additives (control) or treated with essential oils at the rate of 120 or 240 mg kg^{-1} DM. Essential oils used were cinnamon (Cinn120 or Cinn240, respectively), thyme (Thy120 or Thy240, respectively), mint (Mint120 or Mint240, respectively), oregano (Oreg120 or Oreg240, respectively) and cumin (Cum120 or Cum240, respectively). Each treatment was replicated in a quadruplicate manner. All additives were dissolved in 0.5 v/v aqueous ethanol (Chaves *et al.* 2012) and sprayed onto the chopped forages. The same amount of the ethanol was also added to the control.

Aerobic stability

After silo opening, 2 kg samples of each replicate from each treatment were placed loosely into a clean, 20 L bucket. Silages were exposed to air at room temperature and thermometers were placed in the center of the silage masses. A double layer of cheesecloth was placed over each container to prevent drying and contamination, but allowing penetration of air. Ambient temperature and the temperature from each silage were recorded every 2 h. Temperatures were monitored for 12 days. Aerobic stability was defined as the number of hours the silage remained stable before a 2 ˚C increase in temperature above the ambient temperature (Moran *et al*. 1996).

Gas production technique

One liter of rumen liquor was obtained before the morning feeding from two ruminally fistulated lactating Holstein dairy cows. Animals were fed (g/kg, DM basis): alfalfa hay, 240; corn silage, 160; steam flaked corn grain, 210; whole linted cottonseed, 120 and a barley grain-based concentrate, 270 [19% crude protein (CP), 34% neutral detergent fiber (NDF) and 1.6 Mcal/kg NEl]. Pooled rumen fluid was squeezed through 4 layers of cheesecloth into an insulated thermos.

Under continuous flushing of CO_2, 30 mL of buffered rumen fluid (ratio of buffer to rumen fluid was 2:1, buffer were prepared as proposed by Menke and Steingass, 1988) was dispensed with pump into a 125 mL serum bottle containing 250 mg dried of each experimental silage. Eight bottles were considered for each treatment in two separate runs. Bottles were sealed with rubber stopper and aluminum cap and placed in a shaking water bath for 24 h at 38.6 ˚C. At the end of incubation, gas pressure was measured by a pressure transducer and converted into volume using an experimentally calibrated curve (Theodorou *et al.* 1994).

Methane production was determined using a multiple gas detector (SR2-BIO System, SEWERIN, Germany).

Finally, the content of each bottle were filtered and the residue was oven dried (65 ˚C for 48 h) and used to calculate *in vitro* dry matter disappearance (IVDMD). Partitioning factor (PF) was calculated as mg DM disappeared per ml accumulative gas produced within 24 hours post incubation.

Chemical composition

Dry matter content of the silages was determined by air forced oven (65 ˚C for 48 h). Dried silage samples were ground to pass through a 1 mm-screen for later analysis. Crude protein was measured according to the Kjeldahl procedure (AOAC, 1990) on the Tecator Auto-analyzer (Kjeltec 2300 Auto-analyzer, Foss Tecator AB, Hoganas, Sweden).

Determination of NDF was done according to Van Soest *et al.* (1991). Aqueous extract were prepared from ensiled samples by mixing 50 g of forage with 450 mL of deionized water and homogenizing this mix for 1 min. Then, silage pH was determined using a portable pH meter. A portion of aqueous extracts were filtered through four layers of cheesecloth and acidified with 0.2 N HCl (1:1). NH_3-N concentration of acidified silage extracts were determined using distillation method.

Statistical analysis

The effects of type of essential oils regardless of applied level (mathematical mean of both levels) and also different levels of each essential oil compared with the control were tested separately using general linear models (GLM) procedure of SAS (2002). The model used for each of the analysis was as follows:

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

Where:
Y_{ij}: dependent variable.
μ: population mean for the variable.
T_i: effect of treatment i.
e_{ij}: random error associated with the observation ij.

When the overall F-test was significant, differences between means and the control were declared significant at ($P<0.05$) using the Dunnett comparison test.

RESULTS AND DISCUSSION

Chemical composition, pH, NH_3-N concentration (mg dL^{-1}) and aerobic stability of the experimental silages based on the type of applied essential oils are shown in Figure 1 and Table 1. Higher ($P<0.05$) DM content was observed in Cinn120 or Cinn240 compared with the control (Table 1). Relative to the control, CP concentration of silages supplemented with mint or oregano essential oils increased significantly ($P<0.05$). Application of thyme essential oil significantly suppressed NDF of silage compared with the control ($P<0.05$). Cinn240 and Cum240 also had lower NDF concentration compared with the control ($P<0.05$). Furthermore, significant increment in NDF concentration of Mint120 and Oreg240 was observed compared with the control ($P<0.05$). The pH value of Mint120 and Mint240 was significantly higher and lower than the control, respectively ($P<0.05$). Moreover, addition of oregano or cumin essential oils to the corn silage increased its pH value relative to the control ($P<0.05$). A significant reduction in NH_3-N concentration of silages treated with thyme essential oil was observed in comparison to the control. On the other hand, NH_3-N concentration of Mint120, Cum240, Oreg120 and Oreg240 were arisen compared with the control ($P<0.05$). All of applied essential oils except of mint caused a significant improvement in silage aerobic stability compared with the untreated silage (ranging from 44% to 400% increment).

Total gas and methane production, IVDMD and PF of the experimental silages based on the type of applied essential oils are shown in Figure 2. In addition, all of these parameters are shown in Table 2 based on the different levels of each essential oil. Relative to the control, only total produced gas from Oreg240 was diminished ($P<0.05$) and Cinn240, Thy120, Thy240 or Cum120 produced more gas ($P<0.05$) 24 h post incubation (Table 2). When compared with the control, all treated silages except of Mint240 or Oreg120 produce higher methane after 24 h incubation ($P<0.05$). However, the ratio of methane to total gas was not affected by experimental treatments ($P>0.05$). Compared with the control, IVDMD of Thy120, Mint240 or Oreg240 increased by 10 percent ($P<0.05$). On the contrary, Cinn120, Oreg120, Cum120 or Cum240 had lower IVDMD compared with the control. When compared with the control, an improvement was observed in PF of Oreg240, whereas it was reduced in silages treated with cinnamon or cumin essential oils ($P<0.05$).

Silages treated with cinnamon essential oil were characterized by higher DM content. This phenomenon could be due to the limitation of development of special groups of microorganism and therefore smaller loss of nutrients (Selwet, 2009). In the current study, silages treated with mint or oregano essential oils had higher CP concentration. Protein degradation in silage is consequent of proteolytic microorganisms, such as clostridia or enterobacteria present on the plant (McDonald *et al.* 1991). Inhibitory effects of some essential oils including oregano on growth of clostridia were reported in previous studies (Ismaiel and Pierson, 1990).

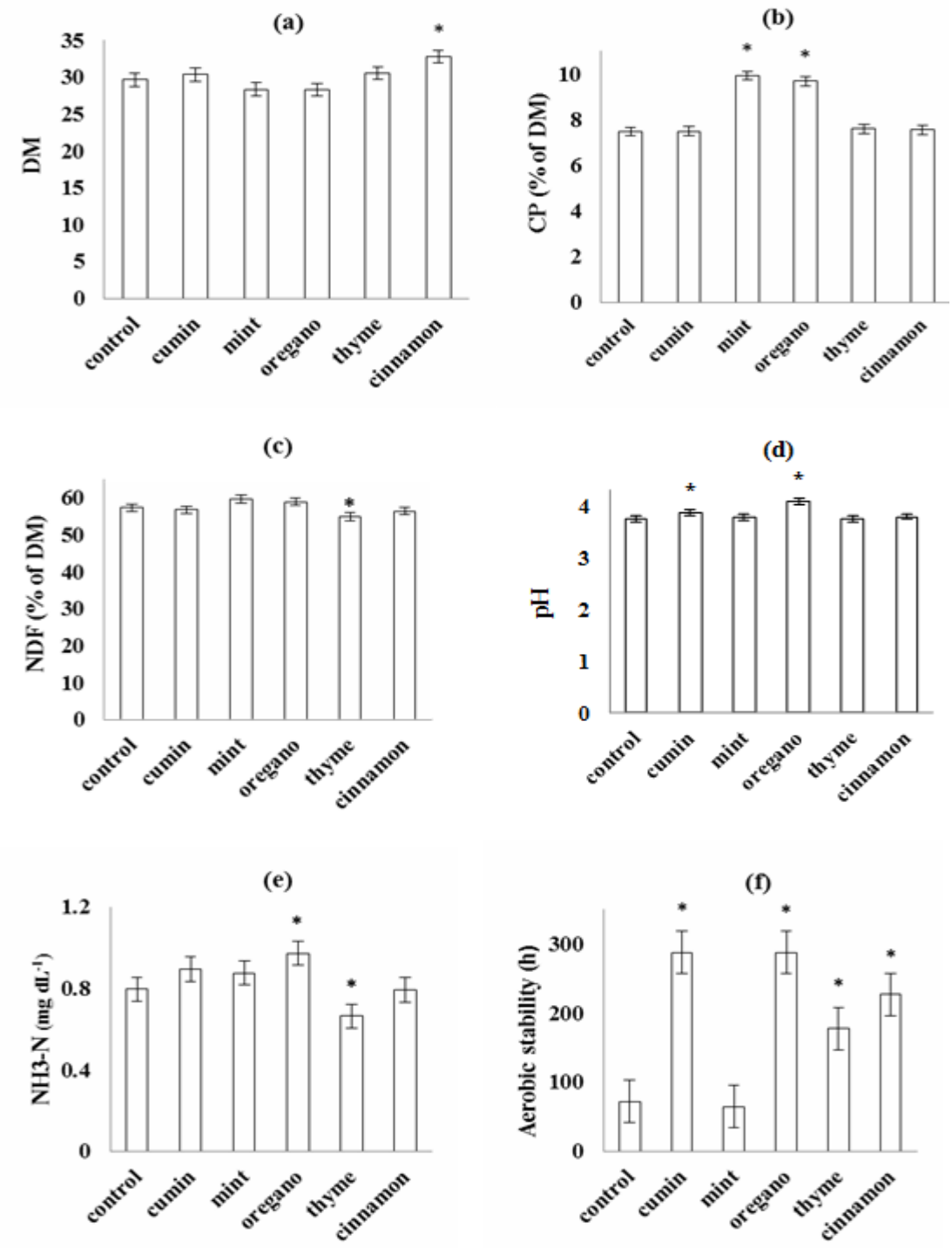

Figure 1 Chemical composition, fermentation characteristics and aerobic stability of corn silages treated with different essential oils (without consideration of applied levels). [(a) dry matter, (b) crude protein, (c) neutral detergent fiber, (d) pH, (e) ammonia nitrogen and (f) aerobic stability]

* Bars with asterisk differ significantly from the control (P<0.05)

On the other hand, higher CP content of silages containing oregano essential oils did not confirm findings of Chaves *et al.* (2012) who observed no change in CP of barley silage supplemented with oregano. This is because of differences in the applied concentrations and possibly the type of silages (Hart *et al.* 2008). Higher pH value in most of the treated silage could be the result of reduction in activity of *Lactobacillus* bacteria, as lactic acid production by these species could decline the pH of the silage (Kung and Ranjit, 2001). However, in two previous studies, essential oils had no inhibitory effect on lactic acid producing bacteria (Kung *et al.* 2008; Chaves *et al.* 2012).

Table 1 The effects of different essential oil on overall composition of corn silage

Chemical composition	Treatments											SEM	P-value
	Control	Cinn120	Cinn240	Thy120	Thy240	Mint120	Mint240	Oreg120	Oreg240	Cum120	Cum240		
DM	29.68	33.51*	32.07*	31.17	29.98	28.32	28.39	26.16*	30.48	29.53	31.18	0.97	0.0023
CP (% of DM)	7.47	7.54	7.54	7.52	7.65	9.76*	10.05*	9.06*	10.26*	7.17	7.79	0.24	< 0.0001
NDF (% of DM)	57.40	57.70	55.30*	55.00*	57.00	63.20*	58.00	57.80	60.20*	58.10	54.20*	0.51	0.0001
pH	3.75	3.77	3.81*	3.73	3.76	4.03*	3.63*	4.06*	4.13*	3.89*	3.86*	0.03	0.0001
Ammonia-N (mg dL^{-1})	0.79	0.82	0.76	0.70*	0.61*	0.90*	0.83	1.06*	0.87*	0.79	0.99*	0.02	0.0113
Aerobic stability (h)	72	165.33*	288*	288*	104*	57	69.33	288*	288*	288*	288*	14.00	< 0.0001

* (P<0.05).

Control: corn silage with no additive; Cinn120: corn silage treated with 120 mg cinnamon essential oil per kg dry matter; Cinn240: corn silage treated with 240 mg cinnamon essential oil per kg dry matter; Thy120: corn silage treated with 120 mg thyme essential oil per kg dry matter; Thy240: corn silage treated with 240 mg thyme essential oil per kg dry matter; Mint120: corn silage treated with 120 mg mint essential oil per kg dry matter; Mint240: corn silage treated with 240 mg mint essential oil per kg dry matter; Oreg120: corn silage treated with 120 mg oregano essential oil per kg dry matter; Oreg240: corn silage treated with 240 mg oregano essential oil per kg dry matter; Cum120: corn silage treated with 120 mg cumin essential oil per kg dry matter and Cum240: corn silage treated with 240 mg cumin essential oil per kg dry matter.

DM: dry matter; CP: crude protein and NDF: neutral detergent fiber.

SEM: standard error of the means.

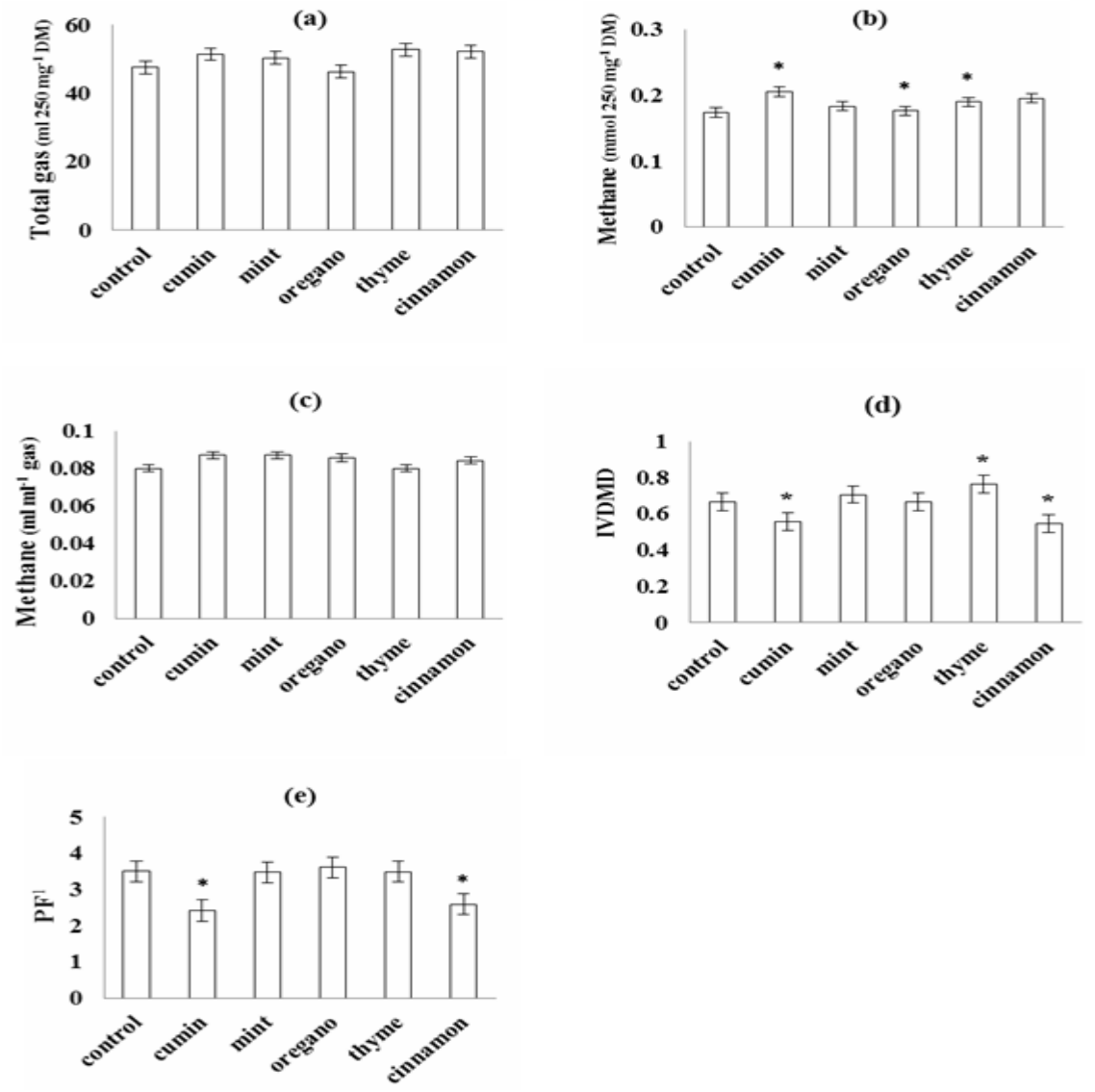

Figure 2 *In vitro* ruminal gas production characteristics of corn silages treated with different essential oils (without consideration of applied levels) after 24h incubation. [(a) total gas production, (b and c) methane production, (d) *in vitro* dry matter disappearance and (e) partitioning factor]

[1] mg DM disappeared/mL gas produced

* Bars with an asterisk differ significantly from the control (P<0.05)

Table 2 *In vitro* ruminal fermentation of corn silages treated with different essential oils after 24 h of incubation

In vitro measurements	Treatments											SEM	P-value
	Control	Cinn120	Cinn240	Thy120	Thy240	Mint120	Mint240	Oreg120	Oreg240	Cum120	Cum240		
Total gas (mL 250 mg^{-1} DM)	48.73	49.93	52.83*	53.08*	54.70*	47.50	49.33	50.00	44.23*	55.13*	47.60	0.711	0.0039
Methane (mmol 250 mg^{-1} DM)	0.174	0.218*	0.201*	0.202*	0.181*	0.185*	0.180	0.187*	0.169	0.218*	0.193*	0.003	0.0499
Methane (mL ml^{-1} gas)	0.080	0.086	0.083	0.083	0.080	0.087	0.085	0.096	0.085	0.086	0.086	0.002	0.1275
IVDMD	0.66	0.58*	0.70	0.73*	0.79*	0.67	0.73*	0.59*	0.73*	0.59*	0.50*	0.027	0.0336
PF	3.49	1.98*	3.19*	3.44	3.53	3.53	3.41	3.15*	4.06*	2.69*	1.86*	0.140	0.0041

* ($P<0.05$).
Control: corn silage with no additive; Cinn120: corn silage treated with 120 mg cinnamon essential oil per kg dry matter; Cinn240: corn silage treated with 240 mg cinnamon essential oil per kg dry matter; Thy120: corn silage treated with 120 mg thyme essential oil per kg dry matter; Thy240: corn silage treated with 240 mg thyme essential oil per kg dry matter; Mint120: corn silage treated with 120 mg mint essential oil per kg dry matter; Mint240: corn silage treated with 240 mg mint essential oil per kg dry matter; Oreg120: corn silage treated with 120 mg oregano essential oil per kg dry matter; Oreg240: corn silage treated with 240 mg oregano essential oil per kg dry matter; Cum120: corn silage treated with 120 mg cumin essential oil per kg dry matter and Cum240: corn silage treated with 240 mg cumin essential oil per kg dry matter.
IVDMD: *in vitro* dry matter disappearance after 24 h incubation.
PF: partitioning factor (mg DM disappeared per mL gas produced after 24 h incubation).
SEM: standard error of the means.

Moreover, pH of Mint240 favorably diminished. To our knowledge, no study has demonstrated the effect of mint essential oil on silage fermentation and its microbial population directly, although stimulatory effect of mint essential oil on growth of some strain of *Lactobacillus* bacteria was reported under *in vitro* condition (Voosogh *et al.* 2009). Results attained from the present study demonstrated that when silage treated with thyme essential oil, NH_3-N concentration decreased indicating inhibition of deamination. Foskolos *et al.* (2010) reported a reduction in NH_3-N concentration of ryegrass silage with high dose of thyme essential oil. Furthermore, McIntosh *et al.* (2003) illustrated that commercial blend of essential oil containing thymol (main active compound of thyme) reduced the rate of deamination under *in vitro* condition. Higher aerobic stability of treated silages confirmed findings of Chaves *et al.* (2012). In their experiment, silages treated with oregano or cinnamon leaf essential oils at 120 mg kg^{-1} DM remained stable for two weeks. Some PSMs such as carvacrol or thymol are able to inhibit the growth of some species of yeasts that are closely associated with aerobic spoilage (Knowles and Roller, 2001; Curtis *et al.* 1996).

In addition to the direct effects of essential oils on silage fermentation, the current study also investigated the possibility of indirect manipulation of ruminal fermentation and methane production by using silages treated with essential oils.

The chemical composition of silage can influence on the rumen microbial fermentation patterns and methane emissions (Navarro-Villa *et al.* 2013). Furthermore, some of essential oils have a good potential to alter rumen microbial fermentation and specially reducing rumen methanogenesis activity (Jahani-Azizabadi *et al.* 2011; Benchaar and Greathead, 2011) and ammonia producing bacteria in the rumen (McIntosh *et al*. 2003; Patra and Yu, 2014).

Gas production of Oreg120 did not alter under *in vitro* condition that is similar to data which already reported by Chaves *et al.* (2012). However, it was decreased in Oreg240 after 24 h incubation. Some of previous studies investigating the effect of oregano essential oils on *in vitro* ruminal fermentation directly, revealed its wide-range spectrum of antimicrobial activity (Dorman and Deans, 2000; Walsh *et al.* 2003). Furthermore, effects of essential oils on rumen microbial populations are dose-dependent (Macheboeuf *et al.* 2008). Higher methane production per incubated DM in most of the treated silages was observed. These data were in contrast with findings of Chaves *et al.* (2012), who found no effect of some essential oils (when used as silage additives) on *in vitro* methane production. This inconsistency may be related to the type and dose of applied essential oils and type of substrate (Jahani-Azizabadi *et al.* 2014). Methane and total gas production had similar changes in each treatment and therefore, the ratio between them was not affected by experimental treatments. Increases in methane production in Cinn120, Oreg120, Cum120 and Cum240 were concomitant with decrease in IVDMD. Beauchemin and McGinn (2006) reported an adverse relationship between methane production and substrate degradation. However, lack of effects on substrate disappearance in response to some plant extracts that have affected methane production has also been reported (Sliwinski *et al.* 2002).

In contrast, a reduction in IVDMD by application of cinnamon, cumin or oregano essential oils has also been reported (Jahani-Azizabadi *et al.* 2011). Oreg240 improved rumen fermentation as reflected by increase in PF. Higher PF in this treatment was consistent with its lower gas production and higher degradation of DM. Roy *et al.* (2014) also reported positive effect of higher dose levels of oregano essential oils on PF.

CONCLUSION

In conclusion, some of essential oils which used as silage additives had desirable effects on chemical composition of corn silage. Mint essential oils at 240 mg kg^{-1} DM had a protective influence on silage protein against deleterious deamination by decreasing pH of the silage. Furthermore, most of the applied additives improved aerobic stability of corn silage noticeably. Moreover, data obtained from the gas production expriment demonstrated the potential of oregano essential oil when used at 240 mg kg^{-1} DM to promote fermentation efficiency through reduction of gas production and increasing *in vitro* dry matter degradability.

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REFERENCES

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Balandrin M.F. and Klocke J.A. (1985). Natural plant chemicals: sources of industrial and medicinal materials. *Science*. **228,** 1154-1160.

Beauchemin K.A. and McGinn S.M. (2006). Methane emissions from beef cattle: effects of fumaric acid, essential oil, and canola oil. *J. Anim. Sci.* **84,** 1489-1496.

Benchaar C., Chaves A.V., Fraser G.R., Wang Y., Beauchemin K.A. and McAllister T.A. (2007). Effects of essential oils and their components on *in vitro* rumen microbial fermentation. *Canadian J. Anim. Sci.* **87,** 413-419.

Benchaar C. and Greathead H. (2011). Essential oils and opportunities to mitigate enteric methane emissions from ruminants. *Anim. Feed Sci. Technol.* **166,** 338-358.

Busquet M., Calsamiglia S., Ferret A. and Kamel C. (2005). Screening for the effects of natural plant extracts and secondary plant metabolites on rumen microbial fermentation in continuous culture. *Anim. Feed Sci. Technol.* **123,** 597-613.

Chaves A.V., Baah J., Wang Y., McAllistera T.A. and Benchaar C. (2012). Effects of cinnamon leaf, oregano and sweet orange essential oils on fermentation and aerobic stability of barley silage. *J. Sci. Food Agric.* **92,** 906-915.

Curtis O.F., Hetty K.S., Cassagnol G. and Peleg M. (1996). Comparison of the inhibitory and lethal effects of synthetic versions of plant metabolites (anethole, carvacrol, eugenol and thymol) on food spoilage yeast *(Debaromyces hansenii)*. *Food Biotechnol.* **10,** 55-73.

Dorman H.J.D. and Deans S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* **88,** 308-316.

Dunière L., Sindou J., Chaucheyras-Durand F., Chevallierd I. and Thévenot-Sergentet D. (2013). Silage processing and strategies to prevent persistence of undesirable microorganisms. *Anim. Feed Sci. Technol.* **182,** 1-15.

Duniere L., Gleizal A., Chaucheyras-Durand F., Chevallier I. and Thevenot-Sergentet D. (2011). Fate of *Escherichia coli* O26 in corn silage experimentally contaminated at ensiling, at opening or after aerobic exposure and protective effect of various bacterial inoculants. *Appl. Environ. Microbiol.* **77(24),** 8696-8704.

Foskolos A., Cavini S., Rodriques-Prado M., Ferret A. and Calsamiglia S. (2010). A screening test of the use of essential oils compounds on ryegrass silage for preventing nitrogen losses in sustainable dairy production systems. Pp. 451-452 in Proc. 3rd EAAP Int. Symp. Energy and Protein. Metabol. Nutr. Parma, Italy.

Fraser G.R., Chaves A.V., Wang Y., McAllister T.A., Beauchemin K.A. and Benchaar C. (2007). Assessment of the effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems. *J. Dairy Sci.* **90,** 2315-2328.

Hart K.J., Yanez-Ruiz D.R., Duval S.M., McEwan N.R. and Newbold C.J. (2008). Plant extracts to manipulate rumen fermentation. *Anim. Feed Sci. Technol.* **147,** 8-35.

Ismaiel A. and Pierson M.D. (1990). Inhibition of growth and germination of *C. botulinum* 33A, 40B, and 1623E by essential oil of species. *J. Food Sci.* **55,** 1676-1678.

Jahani-Azizabadi H., Danesh-Mesgaran M., Vakili A.R. and Rezayazdi K. (2014). Effect of some plant essential oils on *in vitro* ruminal methane production and on fermentation characteristics of a mid-forage diet. *J. Agric. Sci. Technol.* **16,** 1543-1554.

Jahani-Azizabadi H., Danesh-Mesgaran M., Vakili A.R., Rezayazdi K. and Hashemi M. (2011). Effect of various semi-arid native medicinal plant essential oils on rumen fermentation characteristics of a high forage diet using *in vitro* batch culture. *African J. Microbiol. Res.* **27,** 4812-4819.

Khorrami B., Vakili A.R., Danesh Mesgaran M. and Klevenhusen F. (2015). Thyme and cinnamon essential oils: potential alternatives for monensin as a rumen modifier in beef production systems. *Anim. Feed Sci. Technol.* **200,** 8-16.

Knowles J. and Roller S. (2001). Efficacy of chitosan, carvacrol and a hydrogen peroxide-based biocide against foodborne microorganisms in suspension and adhered to stainless steel. *J. Food Prot.* **64,** 1542-1548.

Kung L.J. and Ranjit N.K. (2001). The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. *J. Dairy Sci.* **84,** 1149-1155.

Kung L.J., Williams P., Schmidt R.J. and Hu W. (2008). A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. *J. Dairy Sci.* **91,** 4793-4800.

Losa R. (2001). The use of essential oils in animal nutrition. In: Feed Manufacturing in the Mediterranean Region. Improving Safety: From Feed to Food. Pp. 39-44 in Proc. 3rd Conf. Feed Manufactur. Mediterranean. Reus, Spain.

Macheboeuf D., Morgavi D.P., Papon Y., Mousset J.L. and Arturo-Schaan M. (2008). Dose–response effects of essential oils on *in vitro* fermentation activity of the rumen microbial

population. *Anim. Feed Sci. Technol.* **145,** 335-350.

McDonald P., Henderson A.R. and Heron S.J.E. (1991). The Biochemistry of Silage. Chalcombe Publications, Bucks, UK.

McIntosh F.M., Williams P., Losa R., Wallace R.J., Beever D.A. and Newbold C.J. (2003). Effects of essential oils on ruminal microorganisms and their protein metabolism. *Appl. Environ. Microbiol.* **69,** 5011-5014.

Menke K.H. and Stenigass H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.* **28,** 6-55.

Moran J.P., Weinberg Z.G., Ashbell G., Hen Y. and Owen T.R. (1996). A comparison of two methods for the evaluation of the aerobic stability of whole crop wheat silage. Pp. 162-163 in Proc. 11st Int. Silage Conf. Aberystwyth, UK.

Nanon A., Suksombat W. and Yang W.Z. (2014). Effects of essential oils supplementation on *in vitro* and *in situ* feed digestion in beef cattle. *Anim. Feed Sci. Technol.* **196,** 50-59.

Navarro-Villa A., O'Brien M., López S., Boland T.M. and O'Kiely P. (2013). *In vitro* rumen methane output of grasses and grass silages differing in fermentation characteristics using the gas-production technique (GPT). *Grass Forage. Sci.* **68(2),** 228-244.

Newbold C.J., McIntosh F.M., Williams P., Losa R. and Wallace R.J. (2004). Effects of a specific blend of essential oil compounds on rumen fermentation. *Anim. Feed Sci. Technol.* **114,** 105-112.

Patra A.K. and Yu Z. (2014). Effects of vanillin, quillaja saponin, and essential oils on *in vitro* fermentation and protein-degrading microorganisms of the rumen. *Appl. Microbiol. Biotechnol*. **98,** 897-905.

Roy D., Tomar S.K., Sirohi S.K., Kumar V. and Kumar M. (2014). Efficacy of different essential oils in modulating rumen fermentation *in vitro* using buffalo rumen liquor. *Vet. World.* **7(4),** 213-218.

SAS Institute. (2002). SAS®/STAT Software, Release 9.2. SAS Institute, Inc., Cary, NC. USA.

Selwet M. (2009). Effect of propionic and formic acid mixtures on the fermentation, fungi development and aerobic stability of maize silage. *Polish J. Agron.* **1,** 37-42.

Sliwinski B.J., Soliva C.R., Machmuller A. and Kreuzer M. (2002). Efficacy of plant extracts rich in secondary constituents to modify rumen fermentation. *Anim. Feed Sci. Technol.* **101,** 101-114.

Theodorou M.K., Williams B.A., Dhanoa M.S., McAllan A.B. and France J. (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Technol.* **48,** 185-197.

Van Soest P.J., Robertson J.B. and Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **74,** 3583-3597.

Voosogh A.S., Khomeiri M., Kashani Nijad M. and Jafari S.M. (2009). Effects of mint extract on the viability of probiotic bacteria in a native Iranian dairy drink (Doogh). *J. Agric. Sci. Natur. Resour.* **16(1),** 156-165.

Walsh S.E., Maillard J.Y., Russell A.D., Catrenich C.E., Charbonneau D.L. and Bartolo R.G. (2003). Activity and mechanisms of action of selected biocidal agents on gram-positive and negative bacteria. *J. Appl. Microbiol.* **94,** 240-427.

Yitbarek M.B. and Tamir B. (2014). Silage additives: a review. *Open J. Appl. Sci.* **4,** 258-274.