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Effect of Some Plant Essential Oils on *In vitro* Ruminal Methane Production and on Fermentation Characteristics of a Mid-forage Diet

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ABSTRACT

The objective of the present study was to investigate the effect of different doses of some 7 natural semi-arid medicinal plants' essential oils on *in vitro* ruminal digestion and fermentation patterns of a mid-forage (alfalfa hay: concentrates, 1:1) diet. Treatments consisted of either basal diet alone (control) or added with 35, 70, 140 or 280 µl L^{-1} of coriander, oregano, caraway, cumin, cinnamon, pistachio hull and thyme essential oils, incubated for 24 hours at 38.7ºC. The essential oils of cinnamon and pistachio applied as 280 µl L^{-1} and thyme applied at 140 and 280 µl L^{-1} caused a decrease in DM disappearance as compared with control. Thyme and pistachio essential oils (used at 280 µl L^{-1}) resulted in a decrease of NDF disappearance, while caraway (70 µl L^{-1}) and cumin (140 µl L^{-1}) resulted in an increase in it (14.8% and +18.2%, respectively). Relative to control, the essential oils applied, did not significantly affect the medium N-NH_3 concentration (except thyme at 140 and 280 µl L^{-1}), pH (except thyme and cumin essential oils, 6.41 and 6.22 vs. 6.3, respectively), gas produced (except thyme at 280 µl L^{-1}) and Feed Fermentation Efficiency (FFE). Relative to control, addition of all the essential oils resulted in a decrease of CP disappearance and CH_4 (except for cumin) production as Mm^{-1} incubated. Findings revealed that these essential oils may allow manipulation of rumen microbial fermentation.

Keywords: Essential oil, *In vitro*, Methane, Rumen.

INTRODUCTION

Rumen fermentation includes some such disadvantages as methane emission and ammonia release (Wallace, 2004). In ruminants, methane represents 8 to 12% loss of the intake energy (Johnson and Johnson, 1995). It is a greenhouse gas; bearing a global warming potential of 21 times that of CO_2 (Crutzen *et al.,*1995). Approximately 75-85% of the nitrogen consumed by dairy cows is excreted in feces and in their urine (Tamminga, 1992). Therefore, ruminant nutritionists have long been interested in enhancing energy and protein use efficiency *via* decreasing methane and ammonia nitrogen (N-NH_3) emissions. This has been achieved through optimization of diet formulation and by using such feed additives as growth promoter antibiotics (McGuffey *et al.,* 2001). However, recently have been increased the public concerns on risk of the use of these kinds of additives in animal feeds as regards human health (Official Journal of European Union, 2003). For such reasons, the use of plant extracts and of the Essential Oils (EOs) of medicinal plants, as natural alternatives, has been investigated

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when included in ruminant rations for improving feed efficiency and as well decreasing the adverse environmental effect of ruminants (Wallace, 2004). During the past few years, various studies have been conducted to determine the effects of medicinal plant essential oils on rumen microbial fermentation and on nutrient disappearance (Busquet *et al.,* 2006; Hart *et al.,* 2008). It seems that a concentration of EO in the diet and/or culture to be important as regards its positive effects on rumen microbial fermentation. The Minimum Inhibitory Concentration (MIC) of most EOs is known for common food-spoiling microorganism and pathogens (Burt, 2004). However, there is little information concerning MIC of EOs in complex rumen microbial ecosystem. Authors have reported that EO can affect the activity of the mixture and anoxic culture of rumen microbial population in a dose-dependent manner (McIntash *et al.,* 2003; Busquet *et al.,* 2006; Macheboeuf *et al.,* 2008, Taghavi Nezhad *et al.,* 2011). A wide range of different results have been obtained when various plant essential oils used. The aim of the present study was to investigate the effect of different doses of cinnamon, thyme, pistachio hull, cumin, caraway, oregano and coriander seed essential oils on *in vitro* rumen digestion, fermentation characteristics and efficiency of a 1:1 alfalfa hay: concentrate diet.

MATERIALS AND METHODS

Semi-arid native medicinal plants were collected from Botanical Garden of Ferdowsi University of Mashhad and from some Iranian medicinal herb stores (20 samples per plant) using standard procedure. The essential oil content for each plant was obtained through steam distillation by Celevenger Method. The experimental diet used for batch cultures was a 1:1 alfalfa hay: concentrate [Crude Protein (CP): 17.7%, Neutral Detergent Fiber (NDF): 31.0%, Acid Detergent Fiber (ADF): 21.0%, Ether Extract (EE): 2.7% and Non-Fiber Carbohydrate (NFC): 43%, DM basis]. The diet (DM basis) consisted of alfalfa hay (50.0%), corn grain (17.0%), barley grain (20.5%), sugar beet pulp (3.5%), soybean meal (5.4%) and as well canola meal (3.6%). Samples were oven dried (at 60 ºC for 48h), then ground and passed through 1.5-mm screen. Rumen content was obtained from three adult ruminally fistulated sheep (49.5±2.5 kg, body weight), prior to the morning feeding. Animals were fed 0.6 kg of alfalfa hay and 0.4 kg of concentrate, 24.0 % corn grain, 20.4 barley grain, 27% soybean meal, 13.8% canola meal, 13.8% wheat bran, 0.3% calcium carbonate, 0.2% salt, and as well 0.5% mineral and vitamin premix (each kg containing: 190 g Ca, 90 g P, 50 g Na, 19 g Mg, 3 g Cu, 3 g Fe, 2 g Mn, 3 g Zn, 100 mg Co, 100 mg I, 1 mg Se, 500,000 IU vitamin A, 100,000 IU vitamin D3, 100 mg vitamin E, 3 g antioxidante)]. Ruminal content was immediately strained through some four layers of cheesecloth to eliminate large feed particles and then transferred to the laboratory in a pre-warmed thermos. In an anaerobic condition, 50 ml of buffered rumen fluid [ratio of buffer to rumen fluid 2:1, buffer prepared as proposed by Menke and Steingass (1988)] was dispensed by use of a Pipetor pump into a 125-ml serum bottle containing 0.5 g DM of the experimental diet. Treatments were: control (no additive), cinnamon, thyme, pistachio hull, cumin, caraway, oregano and coriander seed essential oils (6 replicates per each treatment of three runs). Four different doses were taken in the case of each essential oil namely: 35, 70, 140, and 280 µl L^{-1} of the total culture medium. Bottles were sealed with rubber stoppers and aluminum caps, placed in a shaking water bath for 24 hours at 38.6ºC. The essential oils were individually dissolved in ethanol (96%); bottles used as control being dosed the same level of ethanol. In each run, three bottles each receiving merely buffered rumen fluid were taken as blank.

To prevent the overaccumulation of gas produced, head space gas pressure in each

bottle was recorded using a pressure transducer (Theodorou *et al.,* 1994) at 4, 8, 12, 16 and 24 hours past of the incubation, following which gas was released. A sample of the gas was collected into a 10-ml vacuum tube (Venoject®, Terumo Europe N. V., Belgium) at the time of gas pressure determination. Gas pressure was converted into volume using an experimentally calibrated curve. Following 24 hours past of the incubation, the bottles were respectively transferred to refrigerator to stop fermentation and then opened to have medium pH measured using a pH meter (Model 507, Crison Instruments, Alella, Barcelona, Spain), each bottle content being then filtered (42 μm pore size). A 5-ml sample of each bottle filtrate was taken, acidified with 5-ml of 0.2N HCl and frozen at -20ºC. The filtrated residual was oven dried (60ºC for 48 hours) and used to calculate the *In vitro* Dry Matter (IDMD), NDF (INDFD) and Crude Protein (ICPD) Disappearances.

Chemical Analysis

Incubated and/or non-incubated samples were analyzed for Dry Matter (DM), Ether Extract (EE) and Crude Protein (CP) (AOAC, 1995). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined using the method of Van Soest *et al.* (1991).

Nitrogen (N) concentration of non-incubated and *in vitro* incubated samples and as well N-NH_3 concentration of the filtrated bottle content were determined using Kjeldahl Method (Kjeltec 2300 Auto analyzer, Foss Tecator AB, Hoganas, Sweden). Methane content of the produced gas was determined making use of gas chromatography (GC, SRI 8610), Column (Supelco, st. Louis, Mo, USA, 6% cyanopropylphenyl, 94% dimethylpolysiloxane) accompanied by FID detector. Temperatures were adjusted at 100, 200 and 250ºC in column, injector and detector, respectively. Carrier gas (He) flow was adjusted at 24 ml min^{-1}. Methane content was determined through external standard regression curve which had been prepared by use of pure methane gas.

Calculations and Statistical Analysis

Total accumulated gas (ml) and methane [as mmol DM^{-1} incubated and mmol DM^{-1} disappeared (DMD)] produced after 24 hours past of incubation were recorded. The disappearances of DM, CP and of NDF were evaluated as: *Disappearance= (X1- X2)/ X1*, where: *X1*= Incubated (g), and *X2*= The remained after 24 hours past of incubation (g).

Feed Fermentation Efficiency (FFE) was calculated as mg DMD ml^{-1} of accumulative gas produced within 24 hours post incubation. Volumes of methane (ml) were converted to mmol, taking one mol as equivalent to 22.4l L of gas. Data were statistically analyzed using GLM procedure of SAS (V. 8.2) with the statistical model OF: $y=\mu+T_i+e_{ij}$, where y= The dependent variable, μ= Overall mean, T_i= The effect of the essential oil at each concentration level used and e_{ij}= Residual error. Dunnett's test was employed to compare the means with those of the the control ($P< 0.05$).

RESULTS

Effects of natural plant essential oils tested in the present study on *in vitro* ruminal DM, NDF and CP disappearances, of the medium pH and N-NH_3 concentration, total Gas Production (GP), CH_4 production and FFE (g DMD ml^{-1} gas) after 24 hours past of incubation are presented in Tables 1 to 3. Relative to control, the addition of the essential oils did not significantly affect the *in vitro* DM (with the exceptions of: cinnamon, pistachio and thyme essential oils) and NDF disappearance (caraway, cumin, pistachio and thyme essential oils being exception). The addition of high levels of cinnamon and pistachio essential oils

Table 1. Effect of various plant essential oils on *in vitro* ruminal Dry matter (DM), Neutral detergent fiber (NDF) and Crude protein (CP) disappearance of a 50:50 alfalfa hay: concentrate diet and as compared with control**.**

		Nutrients		
	Dose	IDMD [a]	INDFD [b]	ICPD [c]
Control	0.0	0.765	0.445	0.821
Coriander essential oil	35	0.749	-	0.799
	70	0.744	0.403	0.785*
	140	0.742	0.390	0.788
	280	0.727	0.435	0.776*
Oregano essential oil	35	0.748	0.393	0.786*
	70	0.761	0.402	0.778*
	140	0.727	0.418	0.753*
	280	0.730	0.410	0.726*
Caraway essential oil	35	0.748	0.491	0.788
	70	0.761	0.511*	0.834
	140	0.744	0.477	0.792
	280	0.738	0.483	0.784*
Cumin essential oil	35	0.752	0.476	0.791
	70	0.767	0.490	0.826
	140	0.757	0.526*	0.797
	280	0.737	0.473	0.746*
Cinnamon essential oil	35	0.734	0.468	0.797
	70	0.734	0.469	0.785*
	140	0.749	0.481	-
	280	0.705*	0.467	0.780*
Pistachio hull essential oil	35	0.746	0.440	0.773*
	70	0.752	0.459	0.791
	140	0.728	0.466	0.753*
	280	0.715*	0.353*	0.757*
Thyme essential oil	35	0.754	0.415	0.786*
	70	0.740	0.409	0.772*
	140	0.721*	0.385	0.720*
	280	0.600*	0.151*	0.455*
SEM [d]		0.002	0.004	0.001

[a] *In vitro* Dry Matter; [b] *In vitro* NDF disappearance [c] *In vitro* CP disappearance, [d] Standard Error of the Means. * Within a column, means with an asterisk differ significantly from control (P< 0.05).

(280 µl L^{-1}) and as well thyme essential oil (140 and 280 µl L^{-1}) resulted in a decrease (P< 0.05) in IDMD (Table 1). When concentrations of 70 µl L^{-1} of caraway and 140 µl L^{-1} of cumin essential oils were involved, the disappearance of NDF increased significantly (P< 0.05), as compared with that of control (0.511 and 0.526 *vs.* 0.445, respectively). The addition of high levels (280 µl L^{-1}) of both pistachio and thyme essential oils resulted in a decrease in INDFD (Table 1).

Relative to control, the addition of high levels (280 µl L^{-1}) of all the essential oils, resulted in a decrease (P< 0.05) in ICPD (Table 1), ranging between 4.5 and 44.6%, respectively for caraway and thyme essential oils. Results demonstrated that oregano and thyme essential oils were more potential in decreasing ICPD at all their levels applied. Besides, medium N-NH_3 concentration was not affected by the addition of the essential oils applied (except for 140 and 280 µl L^{-1} of thyme essential oil). The addition of 140 and 280 µl L^{-1} of thyme essential oil resulted in a

Table 2. Effect of plant essential oils on medium pH, N-NH_3, and FFE (compared with control) in *in vitro* rumen microbial fermentation of a 50:50 alfalfa hay: concentrate diet.

		Item		
	Dose	pH	N-NH_3 (mg dl^{-1}) [a]	FFE [b]
Control	0.0	6.30	41.3	7.34
Coriander essential oil	35	6.35	41.2	7.03
	70	6.34	42.2	7.21
	140	6.36	41.3	7.39
	280	6.34	40.1	7.61
Oregano essential oil	35	6.34	38.4	7.51
	70	6.35	38.0	7.52
	140	6.35	46.6	6.92
	280	6.34	41.2	7.78
Caraway essential oil	35	6.30	41.0	7.39
	70	6.32	43.1	7.49
	140	6.28	41.3	6.98
	280	6.27	38.7	7.20
Cumin essential oil	35	6.31	43.3	7.50
	70	6.32	44.3	7.55
	140	6.28	44.9	7.55
	280	6.22	43.4	7.50
Cinnamon essential oil	35	6.32	36.1	7.50
	70	6.33	36.8	7.64
	140	6.33	36.2	7.98
	280	6.27	41.1	6.43
Pistachio hull essential oil	35	6.35	40.5	7.93
	70	6.34	41.9	8.27
	140	6.33	40.8	7.33
	280	6.34	47.0	6.74
Thyme essential oil	35	6.27	40.7	7.66
	70	6.30	40.8	7.37
	140	6.27	33.3*	7.81
	280	6.41*	28.1*	7.78
SEM [c]		0.003	0.358	0.041

[a] ammonia nitrogen; [b] Feed fermentation efficiency; [c] Standard Error of the Means. * Within a column, means with an asterisk differ significantly from control (P< 0.05).

reduction (P< 0.05) in N-NH_3 concentration of the media in-contrast with that of the control (33.3 and 28.1 *vs.* 41.3 mg N 100 ml^{-1}, respectively). However, pH of the media after 24 hours past of incubation was not affected by the experimental treatments as compared with control, except for the cases of high levels of cumin (6.22 *vs.* 6.3) and thyme (6.41 *vs.* 6.3). In addition, essential oils, used did not affect Feed Fermentation Efficiencies (FFE) as compared with those of control.

Relative to control, GP (ml 0.5 g^{-1} DM incubated) was not affected by the addition of essential oils, except for 280 µl L^{-1} of thyme essential oil. In addition, all the essential oils, except those from cumin, resulted in a decrease (P< 0.05) in methane production (mM DM^{-1} incubated). The minimum essential oil concentration needed to reduce methane production (mM DM^{-1} incubated) ranged from 35 to 280 µl L^{-1} of the media. The most potentially effective essential oils were observed to be those thyme and oregano, closely followed by cinnamon, pistachio hull, coriander and caraway (Table 3). However, just coriander, oregano and thyme (at 280 µl L^{-1}), caraway (at 35 and 70 µl L^{-1}) and pistachio hull essential oils (at 70 µl L^{-1}), caused decrease

Table 3. Effect of plant essential oils on total gas and on methane production (compared with control) in *in vitro* rumen microbial fermentation of a 50:50 alfalfa hay: concentrate diet**.**

	Dose	Item		
		Gas (ml 0.5 g^{-1} incubated DM 24 h^{-1})	CH_4 (mM DM^{-1} incubated)	CH_4 (mM DM^{-1} incubated)
Control	0.0	104.2	1.83	1.20
Coriander essential oil	35	106.9	-	-
	70	103.2	1.65	1.11
	140	100.4	1.53*	1.03
	280	95.7	1.44*	0.99*
Oregano essential oil	35	99.6	1.62	1.08
	70	101.3	1.6*	1.06
	140	105.5	-	1.19
	280	94.1	1.24*	0.85*
Caraway essential oil	35	101.2	1.51*	1.01*
	70	101.9	1.42*	0.93*
	140	106.7	1.97	1.32
	280	105.3	1.86	1.27
Cumin essential oil	35	100.2	1.70	1.13
	70	101.5	1.78	1.16
	140	101.7	1.61	1.07
	280	105.4	1.79	1.19
Cinnamon essential oil	35	98.7	1.70	1.15
	70	96.6	1.79	1.18
	140	94.1	1.58*	1.05
	280	92.2	1.70	1.20
Pistachio hull essential oil	35	94.0	1.57*	1.05
	70	91.3	1.43*	0.95*
	140	99.9	1.51*	1.04
	280	106.1	-	-
Thyme essential oil	35	98.5	1.69	1.12
	70	100.4	1.73	1.17
	140	92.4	1.58*	1.10
	280	77.2*	1.02*	0.85*
SEM[a]		0.577	0.009	0.008

* Within a column, means with an asterisk differ significantly from control (P< 0.05).
[a] Standard Error of the Means.

(P< 0.05) in mM methane production per disappeared DM (Table 3).

DISCUSSION

In a general look, the present results indicate that a high concentration of medicinal plant essential oils used, resulted in noticeable effect on rumen microbial fermentation, consistent with their antimicrobial activity and previously confirmed in *in vitro* findings (Reuter *et al.,* 1996; Busquet *et al.,* 2006; Bodas *et al.,* 2008). Results attained from the present study demonstrated that when high levels of cinnamon, pistachio hull and thyme essential oils applied, IDMD and INDFD become (cinnamon essential oil being an exception) reduced, confirming the observations of Jahani-Azizabadi *et al.* (2011). It has been demonstrated that natural essential oils of plants bear the kind of antimicrobial activity that may alter energy and protein utilization efficiency in the rumen (Kamel, 2000; Cardozo *et al.,* 2005; Busquet *et al.,* 2004;

Busquet *et al.,* 2006). However, there is limited information as regards the effect of natural plant essential oils (at their different doses) on *in vitro* ruminal microbial fermentation.

Thymol and carvacrol constitute the main active compounds of thyme and oregano essential oils (Dorman and Deans, 2000; Giordani *et al.,* 2004), while cinnamaldehyde and eugenol forming the main active compounds of cinnamon essential oil (Davidson and Naidu, 2000). It has been revealed that these compounds (thymol, carvacrol, cinnamaldehyde and eugenol) possess a wide-range spectrum of antimicrobial activity against gram-positive and gram-negative bacteria (Helander *et al.,* 1998; Dorman and Deans, 2000; Walsh *et al.,* 2003).

Thyme essential oil at its 280 µl L^{-1} reduced INDFD (-66%), GP (-26%) and CH_4 production as mM DM^{-1} disappeared (-29%) and CH_4 production as mM DM^{-1} incubated (-13.6 and -44.2%), and at 140 and 280 µl L^{-1} decreased IDMD (-5.7 AND -21.6%), ICPD (-12.3 and -44.6%) and N-NH_3 concentration (-19.4 and -31.9%). The present results confirm the findings of Jahani-Azizabadi *et al.* (2011), who observed that the addition of 1,000 µl L^{-1} of thyme essential oil reduced IDMD, ICPD and N-NH_3 concentration in a 80:20 forage:concentrate diet. Castillejos *et al.* (2006) investigated the effect of some pure essential oil active compounds on microbial fermentation of a 60:40 forage:concentrate diet in dual-flow continuous culture. They demonstrated that 500 mg L^{-1} of thymol resulted in a decrease in NDF disappearance. The decrease in N-NH_3 concentration with the addition of thyme essential oil was consistent with inhibition proteolysis, peptidolitic and deamination process. McIntosh *et al.* (2003) demonstrated that a commercial blend of essential oil compounds containing thymol reduced the rate of amino acid deamination and inhibited the growth of a specific group of ammonia hyper-producing bacteria (especially *Clostridium Stiklandii* and *Peptostreptococcusanaerobius*). Brochers (1965) and Castillejos *et al.* (2007) observed that an addition of thymol (an active compound of thyme and oregano essential oils) to a medium containing rumen liquid resulted in an accumulation of amino acids nitrogen and a decrease in the ammonia-nitrogen.

Present results indicate that oregano essential oil is of the potential to modify ICPD and *in vitro* ruminal methane production without affecting IDMD, INDFD, media pH, GP, FFE and N-NH_3 concentration; changes being dose depending (Tables 1 to 3). The oregano essential oil resulted to decrease ICPD (-4.3, -5.2, -8.3 and -11.6%, respectively) at 35, 70, 140 and 280 µl L^{-1} , CH4 production as mM DM^{-1} incubated (-12.5 and -32.2%, respectively) at 70 and 280 µl $L^{-1}$and CH4 production as Mm DM^{-1} disappeared (-29.2%) at 280 µl L^{-1} ($P< 0.05$). Jahani-Azizabadi *et al.* (2011) reported that the addition of oregano essential oil resulted in significant ($P< 0.05$) decrease in IDMD, ICPD, N-NH_3 concentration and GP. The inconsistencies between and among some results may be attributed to the different doses of oregano essential oil used, the different ratios of forage: concentrate and as well to the experimental conditions. Busquet *et al.* (2006) evaluated the effect of oregano oil on *in vitro* rumen fermentation of a 50:50 forage: concentrate diet reporting that oregano essential oil at the rate of 300 mg L^{-1} resulted in a decrease ($P< 0.05$) in N-NH_3 concentration, not in agreement with findings of the present study.

Within the course of the present study, thyme and oregano essential oils were shown to decrease methane production per g of DM incubated and disappeared, as compared with those of control, confirming the findings of some previous studies (Ewans and Martin, 2000; Jahani-Azizabadi *et al.,* 2011). Ewans and Martin (2000) observed the addition of 400 mg L^{-1} of thymol to an *in vitro* medium strongly inhibiting the production of methane.

There is limited information concerning the effect of pistachio hull essential oil on rumen microbial fermentation. Results of the present study showed that pistachio essential oil relative to the control resulted to decrease IDMD (-6.5%) and INDFD (-20.7%) at 280 µl L^{-1}, ICPD (-5.8, -8.3 and -7.8%, respectively at 35, 70 and 280 µl L^{-1}), CH4 production as mM DM^{-1} (-14.5, -21.8 and -15.5%, respectively) and CH4 production as mM DM^{-1} disappeared (-20.8%) at 70 µl L^{-1}(P< 0.05). These results confirm the observations of Jahani-Azizabadi *et al.* (2011), who reported that the addition of a high dose (1,000 µl L^{-1}) of pistachio hull essential oil to a high forage: concentrate (80:20) diet resulted in a general inhibition of *in vitro* rumen microbial fermentation. Results obtained from the present study demonstrated that under the prevailing experimental conditions and relative to control, pistachio hull essential oil at all its doses resulted in a decrease in ruminal methane production while this not happening with the other essential oils. Such phenolic compounds as thymol, eugenol and carvacrol are responsible for the antimicrobial activity of many essential oils against either of gram-positive or gram-negative bacteria (Kim *et al.,* 1995; Dorman and Deans, 2000; Lambert *et al.,* 2001). Pistachio hull essential oil carries high concentrations of phenolic compounds (Labavitch *et al.,* 1982). In the previous study the reduction in INDFD was observed when essential oils containing either high concentrations of phenolic compounds (Fraser *et al.,* 2007) or in a pure state of phenolic compounds (Castillejos *et al.,* 2006) were employed.

Cinnamon essential oil at the rate of 280 µl L^{-1} tended to decrease (P< 0.05) IDMD (-7.8%); while at 70 and 280 µl L^{-1} tending to decrease (P< 0.05) ICPD (-4.4 and -5%, respectively), and at 140 µl L^{-1} tended to decrease (P< 0.05) CH_4 production as mM DM^{-1} incubated (-13.7%). These results confirm the findings of Jahani-Azizabadi *et al.* (2011). Fraser *et al.* (2007) demonstrated that an addition of cinnamon leaf oil at 500 mg L^{-1} (when Rumen Simulating Technique employed (RUSITEC) and dual-flow continuous culture system applied) may not affect CH_4 production. The differences observed in these observations may be attributed to the part of the tree used for essential oil extraction (tree hull *vs.* leaf), different methods and as well to the basal diet. Decrease in ammonia nitrogen concentration due to an addition of either cinnamon or cinnamaldehyde have been observed in previous studies (Cardozo *et al.,* 2004; Busquet *et al.,* 2006, Jahani-Azizabadi *et al.,* 2011) in contrast with the findings in the ongoing study. However, Busquet *et al.* (2005) found no effect of these compounds' oils on nitrogen metabolism. The findings obtained from the present study indicate that cinnamon essential oil does not alter INDFD. This result is in contrast with the data reported by Fraser *et al.* (2007), who demonstrated that an addition of a high level (500 mg L^{-1}) of cinnamon leaf oil in dual-flow continuous culture system resulted in a decrease in the NDF disappearance. At least, part of these inconsistencies may be related to the dose used, experimental conditions, methods and as well to basal diets.

A minimum concentration of pistachio hull, cinnamon and thyme essential oils that caused decrease in IDMD under our experimental conditions was 140 µl L^{-1} for the thyme essential oil *vs.* 280 µl L^{-1} for cinnamon and pistachio hull essential oils.

Cumin essential oil at its 280 µl L^{-1} reduced ICPD and pH (-9.1 and -1.3%, respectively) and while at 140 µl L^{-1} increase INDFD (+18.2%). While, caraway essential oil (at 280 µl L^{-1}) tended to decrease (P< 0.05) ICPD (-4.5%); it at 35 and 70 µl L^{-1} tended to decrease (P< 0.05) CH_4 production as mM DM^{-1} incubated (-17.5 and -22.4%, respectively) and at 70 µl L^{-1} tended to increase (P< 0.05) INDFD (+14.8%). Jahani-Azizabadi *et al.* (2011) reported that the addition of high levels (1,000 µl L^{-1}) of cumin and caraway essential oils resulted in a decrease in IDMD, ICPD, N-NH_3 concentration and CH_4 production as mM DM^{-1} disappeared

(38 and 40%, respectively) of a 80:20 forage: concentrate diet. In addition, Jahani-Azizabadi *et al.* (2009) investigated the effect of cumin powder (4% of incubated DM) on *in vitro* rumen microbial fermentation of alfalfa hay reporting a significantly (P< 0.05) decreased GP and methane production compared with those of control (10.81 *vs.* 11.97 and 3.24 *vs.* 3.59 mM DM^{-1} incubated, respectively). Unfortunately, little information is available on the effect of either cumin and caraway essential oils or their powder on rumen fermentation and methane production. The inconsistencies among or between results obtained in different studies may be attributed to the doses used, basal diet and form of cumin and caraway made use of (essential oil *vs.* seed powder).

Some main compounds of coriander essential oil are p-cymene and Linalool (Chao *et al.,* 2000). Coriander essential oil at the rates of 70 and 280 µl L^{-1} tended to decrease (P< 0.05) ICPD (-4.4 and -5.5%, respectively); at 140 and 280 µl L^{-1} tended to decrease (P< 0.05) CH_4 production as mM DM^{-1} incubated (-16.4 and -21.3%, respectively) and at 280 µl L^{-1} tended to decrease (P< 0.05) CH_4 production as mM DM^{-1} disappeared (-17.5%). However, the observed responses were dose depending. Results of the present study demonstrated that under the present study's experimental conditions coriander essential oil is of a strong potential to decrease ICPD and methane production that confirming the observations of Jahani-Azizabadi *et al.* (2011).

Beauchemin and McGinn (2006) observed that reduction in methane production was of diverse relationship with substrate disappearance. Throughout the present experiments coriander and cinnamon (at 140 µl L^{-1}), oregano (at 70 and 140 µl L^{-1}), caraway (at 35 and 70 µl/ L), and pistachio hull essential oils (at 35, 70 and 140 µl L^{-1}) reduced CH_4 (mM DM^{-1} incubated) without IDMD being affected. In addition, an evaluation of the effect of essential oil on CH_4 production, relative to DM disappeared, demonstrated that coriander, oregano and thyme essential oils at their 280 µl L^{-1}, pistachio hull essential oil at 70 µl L^{-1} and caraway essential oil at its 35 and 70 µl L^{-1} reduced methane production. Therefore, these essential oils are proved to increase energy use efficiency, and efficiency related to the disappearing nutrients.

Results of the ongoing study indicate that an addition of the essential oils used, resulted in a decrease of ICPD, without affecting $N\text{-}NH_3$ concentration (except for thyme). $N\text{-}NH_3$ concentration being unaffected and a decrease in ICPD demonstrated that the addition of essential oils causes a decrease of ruminal microbial proteolytic activity stimulating deamination and/or deamination as well as peptidolytic activity. This was because; relative to control $N\text{-}NH_3$ concentration did not change whit decrease in protein degradation.

A reduction in ruminal DM and in NDF disappearance might be nutritionally unfavorable for the animal. But, decrease in ruminal CP disappearance and ammonia-nitrogen concentration might increase ruminal escape of dietary protein and improve the efficiency of nitrogen used in ruminants (Van Nevel and Demeyer, 1988). Consequently, essential oils or their doses that had little negative effect on IDMD and INDFD and resulted in a decrease of ICPD and $N\text{-}NH_3$ concentration may prove more suitable than the other choices.

In conclusion high doses of essential oils, as used in the present study, resulted in their valuable effect on rumen microbial fermentation. In addition to knowledge regarding mechanisms through which these essential oils affect ruminal nitrogen metabolism, there is need to evaluate the effect of the essential oils on concentration of nitrogen sub-fraction. Also, future research is to focus on the optimal doses and on the effect of these essential oils on their *in vivo* rumen microbial fermentation patterns.

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اثر اسانس برخی از گیاهان بر تولید شکمبه ای متان و خصوصیات تخمیر جیره ای با میزان متوسط علوفه در شرایط برون تنی

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چکیده

هدف از این مطالعه بررسی اثر افزودن ۷ نوع اسانس طبیعی گیاهان دارویی مناطق نیمه خشک بر هضم شکمبه ای و الگوی تخمیر جیره ای با میزان متوسط علوفه (علف یونجه خشک: کنسانتره، ۱:۱) در شرایط برون تنی بود. تیمارهای آزمایشی شامل جیره پایه (به عنوان کنترل) ویا جیره پایه به علاوه ۳۵، ۷۰، ۱۴۰ و ۲۸۰ میکرولیتر\ لیتر محیط کشت از اسانس گشنیز، پونه کوهی، زیره سیاه، زیره سبز، دارچین، پوست پسته و آویشن بودند که به مدت ۲۴ ساعت در دمای ۳۸/۷ درجه سانتیگراد کشت داده شدند. اسانس دارچین و پوست پسته به کار رفته در غلظت ۲۸۰ میکرولیتر\ لیتر و اسانس آویشن به کار رفته در غلظت ۱۴۰ و ۲۸۰ میکرولیتر\ لیتر ناپدید شدن ماده خشک را نسبت به تیمار کنترل کاهش دادند. اسانس آویشن و پوست پسته (در غلظت ۲۸۰ میکرولیتر\ لیتر) سبب کاهش ناپدید شدن الیاف نامحلول در شوینده خنثی شدند، در حالی که اسانس زیره سیاه (در غلظت ۷۰ میکرولیتر\ لیتر) و زیره سبز (در غلظت ۱۴۰ میکرولیتر\ لیتر) آنرا افزایش دادند (به ترتیب۱۴/۸+ و ۲/+۱۸ درصد). نسبت به تیمار کنترل، اسانس های به کار رفته اثر معنی داری بر غلظت نیتروژن آمونیاکی محیط کشت (به جز اسانس آویشن در غلظت ۱۴۰ و ۲۸۰ میکرولیتر\ لیتر)، pH (به جز در اسانس آویشن و زیره سبز، به ترتیب ۶/۴۱ و ۶/۲۲ در مقایسه با ۶/۳)، کل گاز تولیدی (به جز غلظت ۲۸۰ میکرولیتر\ لیتر اسانس آویشن) و بازده تخمیر نداشتند. نسبت به تیمار کنترل افزودن اسانس ها سبب کاهش ناپدید شدن پروتئین خام (در دامنه ۴/۴- تا ۴۴/۶- درصد) و تولید متان (به جز در زیره سبز) بصورت میلی مول\ ماده خشک انکوبیت شده (در دامنه ۱۲/۵- تا ۴۴/۲- درصد) شدند اما این کاهش وابسته به غلظت به کار رفته از اسانس بود. نتایج این مطالعه نشان داد که این اسانس ها را می توان به منظور بهبود تخمیر میکروبی در شکمبه به کار برد.