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Full Length Research Paper

Effect of various medicinal plant essential oils obtained from semi-arid climate on rumen fermentation characteristics of a high forage diet using *in vitro* batch culture

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Eighteen natural medicinal plant essential oils and garlic oil obtained from semi-arid climate were conducted to evaluate their effects on *in vitro* rumen microbial fermentation and their effectiveness for decrease *in vitro* ruminal methane production. 50 ml of buffered rumen fluid (1:2; rumen fluid: buffer solution) were introduced in 125 ml serum bottles containing 500 mg of 80:20 alfalfa hay to concentrate as basal diet and or basal diet plus 1 µ/ml of medium of medicinal essential oils or garlic oil (6 replicates for each treatment) and incubated for 24 h at 38.7°C. Head space gas pressure of each bottle was recorded using a pressure transducer at 2, 4, 6, 8, 10, 12, 16 and 24 h of the incubation and a sample was collected to determine methane concentration. In the end of incubation pH measured and samples were collected for ammonia nitrogen concentration and dry matter (DM) and crude protein (CP) disappearance were recorded and feed fermentation efficiency (FFE = mg IDMD/ ml accumulative gas produced at 24 h post incubation) were calculated. Medicinal plant essential oils which caused an increase in FFE and decrease in gas production in-contrast with those in control were selected as the candidates for gas analysis and determine their effects on methane concentration. All essential oils resulted in a significant decrease ($P < 0.05$) in total gas production (except in Rosemary, Dill, Clove, Fennel, Pistachio hull and black pepper) and both IDMD and ICPD (except in Black pepper, Rosemary and Dill). Of the 19 samples tested coriander, rosemary, cinnamon, red basil, oregano 2, black pepper, cumin, caraway and dill that selected as the candidates for gas analysis and determine their effects on methane concentration increase in FFE and decrease in gas production in contrast with those in control. Increase in FFE was more noticeable for thyme, lemon pulp and cinnamon essential oils (22, 21.95 and 3.2 times more than those of control, respectively). Results indicated that coriander, cinnamon, red basil, oregano 2, cumin, caraway and dill essential oils caused a significant decrease ($P < 0.05$) in total methane production (1.5, 0.3, 1.0, 1.3, 1.1, 1.1 and 2.0 compared with 2.3 in control as mmol/g DM incubated, respectively).

Key words: Semi-arid, essential oil, methane, fermentation efficiency, *in vitro*, rumen.

INTRODUCTION

Rumen fermentation includes some disadvantages such as methane emission and ammonia extraction. Methane represents 8 to 12% loss of intake energy (Johnson and Johnson, 1995) in ruminants and it is a greenhouse gas; has a global warming potential 21 times that of CO_2 (Crutzen et al., 1995). Approximately 75 to 85% of nitrogen

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consumed by a dairy cow is excreted in feces and urine (Tamminga, 1992). Ruminant nutritionists have been interested in the long time for enhance energy and protein efficiency with decreasing methane and ammonia nitrogen excretion. This has been achieved through optimization of diet formulation and use of feed additives, such as growth promoter antibiotics (McGuffey et al., 2001), that modify rumen fermentation. Ionophores (monensin, lasalosid, etc.) have been very useful to decrease methane emission and ammonia nitrogen excretion. However, recently there has been increase in public concerns on risk of the use of these kinds of additives in animal feeds for human health. For this reason, the use of plant extracts and essential oils of medicinal plants as natural alternatives has been investigated to include in ruminant rations for improving feed efficiency and decreasing the environmental effect of ruminant animals (Wallace, 2004). During last few years, some studies have been conducted to determine the effects of medicinal plant essential oils on rumen microbial fermentation and nutrient disappearances (Busquet et al., 2006; Hart et al., 2008). However, a wide range of different results have been obtained when various medicinal plant essential oil have been used. The aim of the present study was to investigate the effect of various medicinal plant essential oils obtained from semi-arid climate on *in vitro* rumen fermentation characteristics of a high forage diet.

MATERIALS AND METHODS

Sample collection and experimental diet

Various medicinal plants, obtained from semi-arid climate, were collected from botanical garden of Ferdowsi University of Mashhad and Iranian medicinal plants stores (20 samples per each plant were taken and composited, then a final sample for each plant was taken) using standard procedure. Essential oils content of each plant was obtained with hydro-distillation of grinded samples using Celevenger apparatus. Samples of medicinal plant and effective compounds of their essential oils are presented in Table 1. Experimental diet used for batch cultures was a mixture of alfalfa hay (800 g/ kg) and concentrate (200 g/ kg) which was ground to pass through 1.5 mm screen. The chemical composition of experimental diet is shown in Table 2.

Batch culture and sampling procedure

Rumen fluid was obtained from three adult ruminally fistulated sheep (49.5 ± 2.5 kg, body weight), before the morning feeding. Animals were fed 0.6 kg of alfalfa hay and 0.4 kg of concentrate (24% corn grain, 20.4 barley grains, 27% soybean meal, 13.8% canola meal, 13.8% wheat bran, 0.3% calcium carbonate, 0.5% mineral and vitamin premix, and 0.2% salt). Ruminal content was immediately strained through four layers of cheesecloth to eliminate large feed particles and 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 was 2:1, buffer were prepared as proposed by Menke and Steingass (1988)] was dispensed with pipetor pump into a 125 ml serum bottle containing 0.5 g DM of the experimental diet. Treatments were the basal diet without essential oil (as control) and basal diet plus 1 µl of medicinal plant essential oil or garlic oil per ml of the medium and experiment repeated in two consecutive days (two runs, 6 replicates for each treatment). Then, each bottle was sealed with rubber stopper and aluminum cap and placed in a shaking water bath for 24 h at 38.6ºC. The essential oils were dissolved in ethanol (96%), and the control also dosed the same amount of ethanol. In each run, bottles containing only buffered rumen fluid were provided as blank.

To prevent accumulation of gas produced, head space gas pressure of each bottle was recorded using a pressure transducer (Theodorou et al., 1994) at 6, 8, 10, 12 and 24 h of the incubation and then gas released. A sample of the gas was collected into a 10 ml vacuum tube (Venoject®, Terumo Europe N. V., Belgium) at gas recording times. After 24 h of the incubation, the bottles were respectively transferred to an ice bath to stop fermentation and then opened to measure medium pH using a pH meter (Metrhom pH meter, Model 691). Then, each bottle content was filtered (42 µm pore size) and a 5 ml sample of each filtrate bottle was taken and acidified with 5 ml of 0.2 N HCl and frozen at -20℃. The filtrated residual was oven dried (60℃ for 48 h) and used to calculate *in vitro* dry matter and crude protein disappearances (IDMD and ICPD, respectively).

Chemical analysis

Nitrogen (N) concentration of un-incubated and *in vitro* incubated samples and NH3-N concentration of the bottle content was determined using kjeldahl method (Kjeltec 2300 Auto analyzer, Foss Tecator AB, Hoganas, Sweden). Methane content of the produced gas was determined using gas chromatography (GC, SRI 8610). Temperatures were 100, 200 and 250℃ in column (Supelco, st. Louis, Mo, USA), injector and detector, respectively. Carrier gas-helium (He) flow was adjusted to 24 ml/ min. Methane content was calculated by external standard regression curve which was provided by pure methane gas.

Calculations and statistical analysis

In vitro rumen fermentation characteristics were calculated and reported as total cumulated gas and methane production after 24 h of incubation (as mmol/g DM incubated, mmol/g DM disappeared), methane proportion in total produced gas as mmol methane/mol gas, DM disappearance (as g/g), feed fermentation efficiency (FFE = mg IDMD/ml accumulative gas produced at 24 h post incubation), CP disappearance (as g/g) and medium pH at the end of the incubation. Gas pressure was converted into volume using an experimentally calibrated curve. Data were statistically analyzed using GLM procedure of SAS (1999, V. 8.2) with following statistically model; $y = \mu + T_i + eij$, where y = depended variable, μ = overall mean, T_i = effect of essential oil and e_{ij} = residual error. Dunnett's test was used to compare the means with those of the control ($P < 0.05$).

RESULT

Relative effects (compared to control) of each medicinal plant essential oils on *in vitro* ruminal DM and CP disappearances, gas production and FFE (g DMD/ml gas) after 24 h of the incubation are shown in Table 3. Relative to the control, all essential oils (except in rosemary, dill, clove, fennel, pistachio hull and black pepper) resulted in a significant decrease ($P < 0.05$) in

Table 1. List of various medicinal plants obtained from semi-arid climate, parts used to produce essential oils and their effective compounds.

Formal name	Scientific name	Part of plant	Effective compounds	References
Garlic	*A. sativum*	Bulb	Allicin,diallyl sulfite	Ross et al. (2001)
Cinnamon	*C. zeylanicum*	Bark	Cinnamaldehyde, phenylpropanoid	Chao et al. (2000)
Thyme	*Z. mulrifolra*	Leaves	Thymolz, carvacrol	Bagamboula et al. (2004)
Coriander	*C. sativum*	Seed	Linalol, p-cymene	Chao et al. (2000)
Caraway	*C. carvi*	Seeds	Coumarins	Pitasawat et al. (2007)
Cumin	*C. cyminum*	Seeds	Cuminaldehyde	-
Nutmeg	*M. fragaans*	Seeds	Sabinene, Myristicin,	Muchtaridi et al. (2010)
Dill	*A. graveolens*	Whole plant	Limonene, Carvone	Deans & Ritchie (1987)
Rosemary	*R. officinalis L.*	Leaves	1,8-Cineole, Camphor	Chao et al. (2000)
Red basil	*O. Basilicum*	Whole plant	Estragole	Muller et al. (1994)
Oregano	*O. majorana L.*	Whole plant	Carvacrol, thymol	Kamel (2000)
Oregano	*O. vulgare L.*	Whole plant	Carvacrol, thymol	Kamel (2000)
Mountain pride	*H. persicum*	Flowers	Anethole	Firuzi et al. (2010)
Clove	*S. aromaticum*	Flowers	Eugenol	Kamel (2000)
Lemon	*C. Limon*	Fruits pulp	Limonene	Fisher et al. (2007)
Black pepper	*P. nigrum L.*	Fruits	Sabinene, Limonene, β-Caryophyllene	Chao et al. (2000)
Fennel	*F. vulgare*	Seeds	Anethole	Kamel (2000)
Peppermint	*M. piperitae*	Whole plant	Menthol	
Pistachio	*P. vera*	Fruits hull	-	-

Table 2. Ingredients and chemical composition of experimental diet.

Composition	Amount
Ingredients (% of DM)	
Alfalfa hay	80
Corn grain	6.85
Barley grain	8.10
Sugar beet pulp	1.50
Soybean meal	2.15
Canola meal	1.40
Chemical composition (% of DM)	
Crude protein	17.7
Non fiber carbohydrates	33
Neutral detergent fiber	40.2
Ether extract	2.6
ME (Mcal/kg DM)	2.31

total gas produced. In addition, all essential oils used, except in black pepper, rosemary and dill resulted in a significant reduction ($P < 0.05$) in both IDMD and ICPD. Almost 63.2% of medicinal essential oil used in the present study caused an increase in FFE after 24 h of incubation. These finding was more noticeable for thyme, lemon pulp and cinnamon essential oils (22, 21.95 and 3.2 times over than those of control, respectively). Relative to the control, the addition of coriander, caraway, cumin, nutmeg, thyme, oregano 1, red basil, pistachio hull, clove and mountain pride resulted in a significant increase ($P < 0.05$) of the final pH of the medium (Table 3), while black pepper, rosemary and dill caused a decline in the medium pH ($P < 0.05$). Incontrast to the control, the concentration of ammonia-nitrogen (mg N/100 ml) was significantly ($P < 0.05$) declined when

Table 3. Effect of various medicinal plant essential oils obtained from semi-arid climate on *in vitro* batch culture responses of rumen microbiota including gas production, dry matter and crude protein disappearances and feed fermentation efficiency of a high forage diet.

Variables	Parameters			
	Gas produced (ml/ 0.5 g DM incubated)	DM disappearance	CP disappearance	FFE £
Control	115.7	0.70	0.82	6.09
Coriander	73.7*	0.53 *	0.61 *	7.55
Caraway	78.9*	0.55 *	0.54 *	7.05
Cumin	72.3*	0.53 *	0.53 *	6.92
Fennel	118.7	0.57 *	0.65 *	4.80
Nutmeg	97.7*	0.60 *	0.69 *	6.14
Black pepper	114.7	0.65	0.78	5.52
Thyme	7.3*	0.45 *	0.56 *	134.3*
Oregano 1	71.1*	0.51 *	0.47 *	7.24
Oregano 2	93.3*	0.60 *	0.62 *	6.44
Peppermint	115.0	0.60 *	0.68 *	5.26
Rosemary	116.7	0.65	0.78	5.54
Red basil	76.1*	0.52 *	0.49 *	6.79
Cinnamon	26.6*	0.48 *	0.50 *	19.3*
Pistachio hull	103.1	0.57 *	0.55 *	5.57
Lemon pulp	5.2*	0.44 *	0.56 *	133.6*
Dill	101.8	0.65	0.76	6.39
Clove	112.4	0.34 *	0.16 *	3.05
Mountain pride	86.5*	0.53 *	0.54 *	6.13
Garlic oil	82.6*	0.44 *	0.37 *	5.37
SEM	0.75	0.004	0.005	3.32

Oregano 1= common oregano, oregano 2 = selected from mountain area £ FFE = mg DM disappeared/ ml gas produced after 24 h incubation. * Within a column, means with an asterisk differ significantly from the control (P < 0.05).

cumin, caraway, black pepper, thyme, oregano 1, oregano 2, rosemary, red basil, cinnamon, lemon pulp and dill essential oils and garlic oil were included in the medium (Table 4).

Relative changes (increase or decrease as % of the control value) in gas production and FFE are shown in Figure 1. Based on this information, medicinal plant essential oils represented by the points enclosed within a circle, which caused an increase in FFE and a decrease in gas production incontrast to those of the control, were selected as the candidates to determine methane concentration in the gas produced. These medicinal plant essential oils were coriander, rosemary, cinnamon, red basil, oregano 2, black pepper, cumin, caraway and dill. Results of gas analysis are shown in Table 5. Methane production was expressed as total production (mmol/ g DM incubated); proportion in the gas (mmol/ mol of gas) and relative production per unit of DM disappeared. Results indicated that the selected essential oils caused a significant decrease (P < 0.05) in total methane production (mmol/ g DM incubated), except of rosemary and black pepper which caused an increase (P < 0.05). The addition of coriander, cinnamon, red basil, oregano 2, cumin and caraway also led to a significant lower (P < 0.05) methane production per g DM disappeared by 12.5, 81.2, 37.5, 31.25, 38.1 and 40% as compared with those in control.

DISCUSSION

Finding in this study indicated a potential effect of the medicinal plant essential oils obtained from semi-arid climate used on rumen fermentation characteristics of a high forage diet. In sub-tropical and semi-arid areas of the world, most of the ruminants rely feeding on large quantities of forages systems. Therefore, in these feeding systems, diets have high level of NDF which causes stimulate fermentation to produce ruminal gas with high proportion of methane (Moss et al., 2000). In addition, methane is a greenhouse gas with global warming potential over 21 times more than those in carbon dioxide (Crutzen et al., 1995). Effects of some medicinal plant essential oils and plant extracts on rumen manipulation (e.g. decrease methane production, decrease rumen degradation of dietary protein and etc) have already been reported (Cardozo et al., 2005; Garcia-Gonzalez et al., 2008). It was demonstrated that the effect of each plant

Table 4. *In vitro* effect of various medicinal plant essential oils obtained from semi-arid climate on pH and ammonia-nitrogen concentration of high forage diet through 24 h incubation, using mixed rumen microbiota.

Parameter	pH	Ammonia-nitrogen (mg/dl)
Control	6.56	44.99
Coriander	6.70*	43.73
Caraway	6.64*	33.09*
Cumin	6.68*	32.69*
Fennel	6.60	46.69
Nutmeg	6.66*	44.08*
Blackpepper	6.48*	36.36*
Thyme	6.64*	25.16*
Oregano1	6.62	28.90*
Oregano2	6.63*	34.71*
Peppermint	6.58	42.35
Rosemary	6.46*	38.49*
Redbasil	6.67*	29.72*
Cinnamon	6.58	22.09*
Pistachio hull	6.66*	40.61*
Lemon pulp	6.61	29.34*
Dill	6.50*	30.25*
Clove	6.63*	40.15*
Mountainpride	6.63*	41.34
Garlic oil	6.60	37.91*
SEM	0.005	0.29

Oregano 1 = common oregano, oregano 2 = selected from mountain area. * Within a column, means with an asterisk differ significantly from the control (P < 0.05).

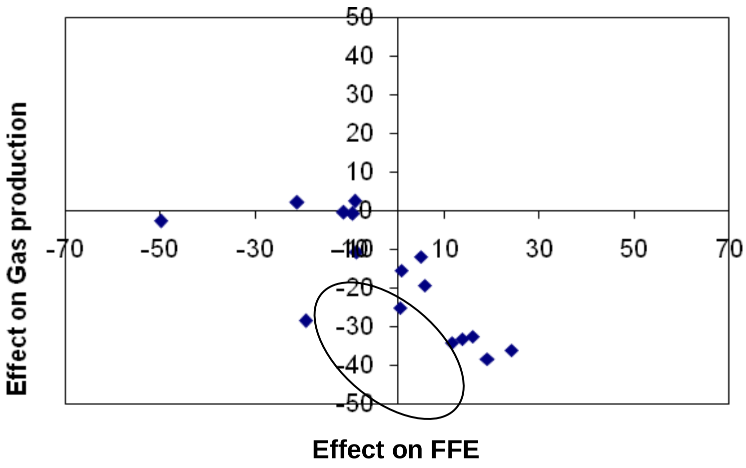

Figure 1. Relative change (percent increase or decrease relative to control) in total gas produced after 24 h of incubation and feed plant essential oils obtained from semi-arid climate. Fermentation efficiency (FFE) in response to addition various medicinal.

species is depended to the nature, concentration and activity of its compounds. Different factors, such as origin, botanical activity, condition of cultivation and growth, harvesting part of the plant used (Wenk, 2003), might influence the conc entration and activity of secondary metabolites within a given plant species. Thus, medicinal plants that are cultivated and grown in semi-arid condition might have different effect on ruminal fermentation and microbial activity.

Our aim of choosing a high dose of the essential oils

(50 µl/ 500 mg of incubated DM) was to supply enough amounts of active compounds for identifying the best semi-arid medicinal plant essential oils. In general looking, present results indicated that a high concentration of medicinal plant essential oils (1 µl/ ml of medium equals 1000 µl/L) resulted in noticeable effect on rumen microbial fermentation which is consistent with their antimicrobial activity and confirmed previous *in vitro* findings (Busquet et al., 2006; Bodas et al., 2008). In addition, results of the present study demonstrated that when supplied a high level of most semi-arid medicinal plant essential oils IDMD disappearance reduced (except in black pepper, rosemary and dill essential oils, compared with those in control). These findings confirm previous results which claimed for a potential of inhibition of essential oils regarding rumen microbial fermentation (Oh et al., 1968).The reduction in ruminal DM disappearance might be nutritional unfavorable for 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 and Demeyer, 1988). In the present study the addition of black pepper, rosemary and dill essential oils to the medium caused a significant decrease ($P < 0.05$) in ammonia-nitrogen concentration while CP disappearance remained unchanged compared with those of control. The lack of the effect of these medicinal essential oils on DM and CP disappearance indicated that the level used was not capable to alter rumen microbial activities.

The gas produced from anaerobic ruminal fermentation is a by-product that accompanied by loss of energy and reduces fermentation efficiency. Therefore, increase in gas production is not suitable at all time. Feed fermentation efficiency is a better index to evaluate fermentation efficiency process, with attention to animal requirement and limitation. To identify possible benefit of medicinal plant essential oils and to select the plants candidate for evaluation methane concentration in produced gas two selection criteria in the present study were applied; decrease in gas production and increase in FFE. Based on this information coriander, rosemary, cinnamon, red basil, oregano 2, black pepper, cumin, caraway and dill essential oils were selected (Figure 1) and their effects on *in vitro* methane production were evaluated.

In agreement with our experiment results, findings of previous experiments reported that several secondary compound from some medicinal plants such as rosemary, garlic, cinnamon, cumin (Jahani-Azizabadi et al., 2009); clove, fennel (Patra et al., 2005); sapindus Spp., populus tremuloides, syzygium zeromatium, terminalia chebula, pisidium guayaba (Kamara et al., 2006); horsetail, sage (Broudiscou et al., 2000); sesbania sesban, acacia angustissima (Zeleke et al., 2005); salix, rheum, quercus, prunus, populus, carduus (Bodas et al., 2008) might decrease methane production from rumen fermentation. Beauchemin and McGinn (2006) observed that a reduction in methane production had adverse relationship with substrate disappearance. However, in our experiment with decline in DM disappearance at the same time, methane production was also decreased (Table 4). It has been shown that 1, 8-cineol, camphor, α and β-pinene are major antimicrobial compounds in rosemary oil (Baratta et al., 1998; Chao et al., 2000; Burt, 2004; Giordani et al., 2004). Information about the effect of the rosemary essential oil on ruminal methane production is very low. Results of the present study are in disagreement with observations of Jahani-Azizabadi (2009). They observed that the *in vitro* addition of rosemary powder resulted in a significant decrease ($P < 0.05$) in methane production compared with those of control.

Main compounds of coriander essential oil are p-cymene and Linalool (Chao et al., 2000). Results of the present study demonstrated that coriander essential oil has a potential to decrease methane production (as mmol/g DM disappeared), while increase FFE. Little information is available about the effect of the coriander essential oil on rumen microbial fermentation and methane production.

Carvacrol and thymol are the main compounds of thyme and oregano essential oils (Giordani et al., 2004). In the present trail, Thyme and Oregano essential oil had a similar effect on ruminal ammonia-nitrogen concentration, IDMD, ICPD and medium pH, as it was indicated in the previous studies (Busquet et al., 2006). Brochers (1965) and Castillejos et al. (2006, 2007) observed that the addition of thymol (active compound of thyme and oregano) to the medium contained rumen liquid resulted in an accumulation of amino acids nitrogen and decrease in the ammonia-nitrogen concentration. Under present study condition, the concentration of methane in produced gas when thyme essential oil applied was very low and it was not detectable with gas chromatography, therefore, results are not present in Table 5. In the present study, oregano essential oil caused a decrease in methane production per g DM incubated and disappeared, and mmol gas produced as 43.0, 31.0 and 28.7%, respectively, in contrast to those of the control. Evans and Martin (2000) observed that addition 400 mg of thymol to one of *in vitro* medium strongly inhibit methane production.

Results of the present study demonstrated that cinnamon essential oil strongly reduced methane production per g DM incubated and disappeared as 87 and 81% relative to those of control, respectively. Reduction in NH_3-N concentration due to the addition of Cinnamon or Cinnamaldehyde was observed in previous studies (Cardozo et al., 2005; Busquet et al., 2006), while Busquet et al. (2005) found no effect on nitrogen metabolism. At least, part of these inconsistencies results may be related to the dose used, experimental condition, methods and basal diets.

Table 5. Effects of some selected medicinal plant essential oils obtained from semi-arid climate on *in vitro* ruminal gas and methane production by mixed rumen microbiota through 24 h incubation.

Fermentation parameter	Control	Coriander	Rosemary	Cinnamon	Red basil	Oregano 2	Black pepper	Cumin	Caraway	Dill	Sem
Gas produced (mmol/gDMincubated)	10.3	6.6*	10.6	2.4*	6.8*	8.3*	10.2	6.9*	7.0*	9.1*	0.056
Gas (mmol/gDMdisappeared)	7.4	6.2	8.1	2.5*	6.6	6.9	8.1	6.5	6.3	7.0	0.078
Methane (mmol/gDMincubated)	2.3	1.5*	3.0*	0.3*	1.0*	1.3*	2.8*	1.1*	1.1*	2.0*	0.017
Methane (mmol/molgas)	2.2	2.2	2.9*	1.3*	1.5*	1.6*	2.7*	1.5*	1.5*	2.2	0.0001
Methane (mmol/gDMdisappeared)	1.6	1.4*	2.3*	0.3*	1.0*	1.1*	2.2*	1.0*	1.0*	1.5	0.0017

Oregano 2 = selected from mountain area. * Within a row, means with an asterisk differ significantly from the control (P< 0.05).

Results of the present study demonstrated that Red basil has a high potential to decrease *in vitro* ruminal methane emission (56% of mmol/ g DM incubated, 37.5% of mmol/ g DM disappeared and 31.5% of mmol/ mol gas produced) and NH3-N (32.7% of mg/ dl) in-contrast with those of control without negative effect on DM and CP disappearances.

In the present study cumin and caraway essential oils had similar effect on fermentation characteristics. Cumin and caraway caused a decrease in methane production as mmol per g of DM disappeared (38 and 40%, respectively) compared with those of control. Jahani-Azizabadi et al. (2009) observed that cumin powder (4% of incubated DM) significantly (P < 0.05) decreased *in vitro* ruminal gas and methane production compared with those of control (10.81 vs. 11.97 and 3.24 vs. 3.59 mmol per g DM incubated, respectively). Unfortunately, little information is available about the effects of cumin and caraway essential oils or powder on rumen fermentation and methane production.

In conclusion some of medicinal essential oils obtained from semi-arid climate have a good potential to manipulate rumen microbial fermentation and specially reducing rumen methanogenesis activity. Results of the present study suggested that the medicinal plant essential oils, such as thyme, dill, cinnamon, cumin and caraway used in the present study has high inhibitory effect on *in vitro* rumen methane production and NH3-N concentration. Therefore, these essential oils might be interest for developing a new additive as an alternative for growth promoter antibiotic. As said above, we choose this dose of essential oil (50 µl/ 500 mg of incubated DM) in order to supply enough amount of active compounds for identifying the best effective medicinal plant essential oils, but as shown in the results for some of essential oils, it's possible that the dose used is much above of the acceptable dose for normal rumen fermentation. So, there is need to evaluate these selected natural additives, using dose-response assay to determine the best effective dose.

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