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Effects of Using *Mentha pulegium* and *Ziziphora clinopodioides* Essential Oils as Additive on *in vitro* study

M. Edalati Nasab[1], A.A. Naserian[2], A.R. Vakili[2] and A.M. Tahmasbi[2]

[1]Ph.D. Student of Ruminant Nutrition, Ferdowsi University of Mashhad International Campus, Mashhad, Iran.
[2]Department of Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

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The purpose of Present study was conducted to evaluate the effects of adding essential oils (*Ziziphora clinopodioides* and *Mentha pulegium*) on alfalfa silage on the rumen degradation parameters with *in vitro* technique. Present study was performed by utilizing an *in vitro* gas production method at various incubation intervals. Rumen fluid taken from three lactation, rumen-fistulated Holstein cows. The gas production rate was measured at standard times from 0, to 96 hours. The outcomes of this experiment show that *Ziziphora clinopodioides* and also *Mentha pulegium* essential oils had a positive influence on gas production rate. Silage content with Mentha pulegium had more decrease effects than *Ziziphora clinopodioides* in Gas production compare with control silage and it was significant. Gas production values (at 96 h incubation) in silage with no added essential oils, 30ML of *Ziziphora clinopodioides* and 30ML of *Mentha pulegium* were 68.82, 56.12 and 49.74, respectively. Compared with control, aerobic stability had a significant difference and it was developed in silage treated with essential oils. The findings of their findings showed that these essential oils could be used to increase the performance of ruminants. In addition, adding essential oils could change the rumen fermentation in ruminant, however, more research is still needed to proving this conclusion.

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Keywords: *Ziziphora clinopodioides*, *Mentha pulegium*, essential oils, Silage

Ruminants have an especial digestive trace that consists microorganisms who degrade feed contents and supply energy and protein for the animals. Ruminant nutritionists have at length been intrigued by modulating those rival microorganisms among different microbial populaces with the destination from claiming moving forward that effectiveness for the vitality of protein utilization in the rumen. This need to be been attained via those streamlining for the formulation of ration and using feed additives which change the environment and upgrade or harness particular microbial populaces (Calsamiglia *et al*. 2006).

According to Calsamiglia *et al*. (2006), for this reason, researchers have ended up intrigued by assessing additives with regulating rumen fermentation, such as the utilization of yeasts, natural acids, plant extracts, probiotics, as well as antibodies. EOs (essential oils) have a great impact on health, for example, on cardiovascular diseases, tumors, inflammation, and the gradual elimination of free radicals (Harborne and

*Corresponding author E-mail: abasalin@yahoo.com

Williams, 2000; Reddy *et al*. 2003; Trouillas *et al*. 2003). These properties rely on upon their capacity to discover free radicals, harness peroxidation of lipids that find a structure of membrane cell. Also, these properties raised the movement of cell reinforcement proteins (Gutierrez et *al*. , 2003; Lee *et al*. 2003). Antiseptics and antimicrobials are considered as the most important activities of these compounds. disinfecting properties of many plants have been known from many years ago. For the first time, Borchers (1965) mention the possible advantage of using essential oils on microbial fermentation in the rumen. Borchers (1965) also in an *in vitro* study viewed that eke out *thymol* to ýrumen fluid caused the aggregation of amino acid Nitrogen (AA-N) and the decreased from claiming Ammodytidae N ýconcentrations, offering that *thymol* prevents amino acid catabolism. After that, Oh *et al*. (1967, 1968) guessed that maybe the slight palatability of some plants by ruminants is related to both of organoleptic effects, and their negative impact on fermentation by rumen microbial as well as digestion of nutrient.

Considering these cases, the purpose of the current research was investigating impacts of two essential oils (*Ziziphora clinopodioides* and *Mentha pulegium*) on rumen fermentation with gas production method.

MATERIALS AND METHODS

Rumen fluid was taken from four lactating, Holstein cows added with rumen-fistulate (body weight 620± 8.9kg; day in milk 45 ± 13). The cows were fed a total blended diet (chemical analysis mention in table 1) containing silage of barley (46.5%), corn (6.8%), silage of hay alfalfa (4.5%), steam rolled barley (17.7%), dairy supplement pellets (24.5%). Formulating The ration to supply nutritious needs of rumen fluid donor cows according to NRC, 2001 nutrition requirement table, and became feeding two times each day (9:00 and 16:00) ad libitum. After achieving Rumen liquid before they fed morning meal, the rumen fluid was filtered with cheesecloth and then transferred into a completely thermo insulated flasks. Because of the method of Menke et al. (1979), a tight anaerobic condition was used in time of rumen fluid collection. Later on, it was transported to the laboratory.

To harvesting Alfalfa forage with 28-30% DM, a New Holland harvester (New Holland North America, New Holland, PA) were used. Chop length might have been situated on accomplishing the cut of 0.95cm. Three piles of chopped forage (10kg chopped forage in each pile) were treated with the following: 1) 0, 10, 20 and 30 mL of *Ziziphora clinopodioides* essential oil, 2) 0, 10, 20 and 30 mL of *Mentha pulegium* essential oil. Alfalfa was ensiled in each trial (500g of DM/kg) from October 1 to November 12, respectively. Silos were stored at 20°C temperatures at the dark and opened 42 days after ensiling.

Aerobic Stability

Each silo was completely blended then 1kg sample was kept from each silo. Each sample transferred to 1 Liter capacity containers (3 containers for each treatment) after growing the silos. Each container has been installed with three Thermochron buttons (Embedded Data Systems, Lawrenceburg, KY) in the top, mid and bottom layers of the silage container to keep the temperature every 20 minutes. Each container was impenetraded with a cheesecloth and kept at the temperature of 20° C up to 7d. Moreover, the temperature of surrounding environment was estimated every 20min at this stage. After 1, 3 and 7 days of aerobic exposure, silages were sampled from each container for chemical investigation, and pH measuring (Tables 2, 3).

Compounds identification

The identification of the parts might have been dependent upon correlation of their mass spectral with those of NIST mass spectral library (Masada, 1976 and NIST, 2002), also, the individuals portrayed by Adams (2001), and comparing their maintenance indices either with those of accurate mixture or with written works qualities (Adams, 2001).

Chemical analysis

Dry matter (DM) specified with drying each sample for 24 hours in an oven drier at 105 ºC, to estimating ash content each sample was burned with muffle furnace, at 500 ºC for 9 hours. Also, by measuring Nitrogen content, utilizing the Kjeldahl method was used (AOAC, 1990). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) estimation were with respect to the Van Soest *et al*. (1991) by applying an ANKOM fiber analyzer. Two EOs were purchased of a commercial

mill in Kashan (Iran). Also, all chemical analyses were repeated in triplicate.

Gas production technique

The *in vitro* gas production procedure was according to the method of Menke *et al*. (1979). Approximately 200mg dry weights of samples (Alfalfa silage non-essential oils and Alfalfa silage with 10, 20 and 30ML of each essential oils) were estimated in triplicate into 100ml glass syringes with respect to the processes of Menke and Steingass (1988).

First of all, each syringe was pre-warmed at 39 ºC, later 30ml of rumen liquor buffer mixture (1:2) injected to all syringes, then heated to 39 ºC in a bain marie. artificial saliva was Prepared with respect to the method of Menke and Steingass (1988). The artificial saliva was made of 237ml buffer solution and 237ml important element solution plus 0.12ml solution of trace element and 1.22 ml resazurin was prepared and stored at 39°C, one day before incubation. Then, the reduction solution (Na2S.9H2O, 0.625g; NaOH 1N, 4ml; distilled water, 95ml) was being added to the incubation of the samples.

The ratio of artificial saliva to ruminal liquor was 2:1 (v/v). In each test and level, three repetitions were utilized, whereas they were mildly shaken for 30min after starting the incubation and for every 1 hour in the first 10h after the incubation. Gas production was evaluated in the calibrated syringes in 2, 4, 6, 8, 12, 16, 24, 48, 72 and 96 hours of incubation.

McIntosh et al. (2003) stated that if levels of essential oils are less than 100 p.p.m, could not change rumen performance. The study of Cardozo (2005) also was in agreement with this findings. In order to specify the impacts of essential oils on the kinetics of gas production on *in vitro*studies, data were adjusted to the Orskov and McDonald (1979) model formula as well as the following:

$Y= a +b (1-e^{-ct})$.

Y= gas product.

a+ b= potential of gas production.

a= rate of gas production of the fast soluble fractions.

b= rate of gas production of the insoluble fractions.

c= constant rate of gas production for the insoluble fractions (ml/h).

t= time of incubation (hours).

Table 1. Analysis of concentrate and forages supply to dairy cows (g/kg DM)

Item	Concentrate	Wheat straw	Alfalfa silage
DM	910.65	89.84	320.55
CP	276.37	44.96	110.42
NDF	275.32	830.11	467.22
ADF	95.51	574.20	289.54
EE	43.22	15.81	30.50
Ash	65.59	87.53	76.48

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; EE: ether extract

Table 2. Composition of alfalfa silage (%) ingredients treated with different doses of Mentha pulegium

Time	Treatment (mg of Mentha pulegium) Control	10	20	30	SEM	P.value
DM	29/10^{d}	32/61^{b}	32/80^{a}	31/25^{c}	0/005	0/001
CP	20/05^{c}	20/20^{b}	20/30^{a}	20/40^{a}	0/02	0/001
NDF	34/20^{b}	33/55^{c}	31/07^{d}	34/37^{a}	0/02	0/001
ADF	26/31^{b}	24/79^{d}	28/10^{a}	26/26^{c}	0/005	0/001
OM	90/50^{c}	89/44^{d}	91/20^{b}	92/06^{a}	0/04	0/001
pH	5/72^{a}	5/59^{a}	5/50^{b}	5/52^{a}	0/06	0/18
Aerobic Stability	86/90^{b}	271/30^{a}	255/25^{a}	269/10^{a}	12/66	0/02

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; OM: organic matter.

a, b, c, d Means significant differences within the row ($p<0.05$).

Statistical Data Analysis

The data of gas production and related parameters were restricted to the one-way analysis of variance by using variance model ANOVA in SAS software (2006). Although, multiple comparison tests or Duncan's multiple-t-test (1980) has been used in similar studies. Significant differences were tested by the multiple range Duncan's method. Mean differences were significant at $p<0.05$ level. The standard error of the means was estimated by using the method of the residual mean square in the analysis of variance. Therefore, all data collected from three times repetition tests (n= 3).

RESULTS

The chemical combination of alfalfa silage, concentrate and wheat straw of ingredients used in the diet of animals, which rumen liquor was taken, is showed in table 1. The chemical composition of alfalfa and their silage (%) treated with various doses of *Mentha pulegium* and *Ziziphora clinopodioides* is seen in table 2 and 3, respectively. As shown in these tables, there were positive differences between silages. In addition, a considerable difference was observed between the forages in terms of chemical composition. According to table 2, the content of crude protein in forages ranged from 20.05 to 20.40%. The silage that treated with 30ML of *Mentha pulegium* had higher crude protein than the other doses of *Mentha pulegium*.

Aerobic stability had a positive difference and it was developed in silage treated with *Mentha pulegium* in comparison with control. The data demonstrates the chemical compositions improved by adding various doses of *Ziziphora clinopodioides* in table 3. pH difference was not positive, but it will diminish slightly in doses of 20 and 30ML of *Ziziphora clinopodioides.*

The effect of incubating the materials *in vitro* during 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours by a various dose of essential oils of *Ziziphora clinopodioides* and *Mentha pulegium* on gas production from the gas test is listed in the tables 4 and 5. The result of the findings indicated adding essential oils on alfalfa silage reduced gas production. It seem reasonable that part of this activity is due to the hydrophobic nature of the cyclic hydrocarbons, which let them associate with cell membranes as well as amass in the Two-layer lipid from bacteria, taking a space between the fatty acids chains (Sikkema *et al*. 1994; Ultee *et al*. 1999). This action and reaction Cause structural changes and changes in the structure of the membrane, resulting in its fluidity and enlargement (Griffin *et al*. 1999). In this situation, lack of membrane stableness brings about the discharge of ions over the cell membranes, that leads to a reduction in the transmembrane ionic difference. In many ways, bacteria can balance these effects with utilizing ionic pumps To prevent cell death, although the great quantity of energy is turned into this mechanism and bacterial growth was restricted (Griffin *et al*. 1999; Ultee *et al*. 1999;

Table 3. Alfalfa silage (%) Chemical composition treated with different doses of Ziziphora clinopodioides

Time	Control	Treatment (mg of Ziziphora clinopodioides) 10	20	30	SEM	P.value
DM	$29/10^{d}$	$29/36^{c}$	$30/64^{b}$	$33/20^{a}$	0/005	0/001
CP	$20/05^{c}$	$20/10^{c}$	$20/18^{b}$	$20/30^{a}$	0/005	0/004
NDF	$34/20^{b}$	$32/50^{d}$	$35/13^{b}$	$38/25^{a}$	0/04	0/002
ADF	$26/31^{b}$	$28/33^{b}$	$24/70^{d}$	$29/65^{a}$	0/02	0/16
OM	$90/50^{c}$	$91/00^{b}$	$91/26^{b}$	$92/19^{a}$	0/29	0/01
pH	$5/72^{a}$	$5/70^{a}$	$5/50^{a}$	$5/51^{a}$	0/1	0/57
Aerobic Stability	$86/90^{b}$	$260/40^{a}$	$239/50^{a}$	$200/00^{b}$	9/11	0/42

DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, OM: organic matter.

a, b, c, d Means significant differences within the row ($p<0.05$)

Cox *et al*. 2001). With respect to increasing the time of incubation, the cumulative content of gas production will be enhanced. According to Table 4, there are significant differences between control treatment and silages treated with various doses of Ziziphora clinopodioides with respect to gas production at each time of incubation. After 96 h incubation, gas produced ranged between 68.82 and 56.12 ml per 200 g of substrate.

Table 5 shows that gas production of silage content with 30ML of *Mentha pulegium* was significantly and positively ($p<0.001$) lower than the other. In addition, as it can be seen, there were positive and significant ($p<0.001$) differences between silages with respect to gas production at all incubation times. Silage content with *mentha pulegium* had more reduction impact than *Ziziphora clinopodioides* in gas production in comparison with control silage and it was significant and positive at ($p<0.001$) level. At 96 h incubation, gas production values of silage no added essential oils, 30ML of *Ziziphora clinopodioides* and 30ML of *Mentha pulegium* were 68.82, 56.12 and 49.74, respectively. All incubation times presented that gas production in experimental silages was lower than control silage, but also silages treated with essential oils had higher protein content than control silage. In alfalfa silage, protein was defectively used, especially when the diet was low in energy. (Buxton, 1996). within harvesting and storage of silage (Albrecht and Muck, 1991), extensive degradation happens, and it was followed by more microbial depression in the rumen (Buxton, 1996). On in vitro study, gas is achieved not only due to fermentation (CO2 and CH4) but also consequentially, from the acidify impact of VFAs (volatile fatty acids) on CO2 which released from bicarbonate buffer solution (Getachew et al. 1998). The breakdown of protein is produced ammonia, combining with H^+ that released from the buffer that remains in the solution produced $NH4^+$ and indirectly inhibits the production of gas. The antimicrobial activity of essential oils in other treatments is one reason that why gas production value of control silage was lower than other treatments. Also, Nagy and Tengerdy (1968) assessed the ruminal microorganisms sensitivity to the essential oils.

Forty three *Mentha pulegium* essential oil compounds were identified, suggesting 99.53% of the total mass of essential oil, of which 29 compounds were clarified. The major components were pulegone (38.83%), menthone (19.24%), pipériténone (16.53%), pipéritone (6.35%) and isomenthone (6.09%), Limonène (4.29%), Octanol (1.85%). The major component of the *Ziziphora clinopodioides* essential oil was monoterpene hydrocarbon and among that, the important ingredients were pulegone (79.34%), Limonene (6.77%) and piperitenone (4.21%). Findings of present study showed that essential oils and doses had positive and significant impacts

Table 4. Gas production in different time incubation of silages treated with Ziziphora clinopodioides

Time	Treatment (mg of Ziziphora clinopodioides) Control	10	20	30	SEM	P.value
2	22/76^{ab}	24/51^{a}	20/02^{c}	19/81^{b}	0/90	0/008
4	31/87^{a}	29/37^{b}	25/84^{c}	22/27^{d}	0/74	0/001
6	37/38^{a}	34/92^{a}	30/98^{b}	27/35^{c}	0/93	0/001
8	43/03^{a}	40/54^{a}	36/94^{b}	35/28^{b}	1/11	0/001
12	48/22^{a}	44/27^{b}	42/34^{bc}	40/59^{c}	1/03	0/001
24	56/40^{a}	48/49^{b}	47/87^{b}	45/72^{b}	1/11	0/001
48	61/16^{a}	52/50^{b}	51/91^{b}	50/02^{b}	0/82	0/001
72	64/02^{a}	56/45^{b}	57/08^{b}	53/49^{c}	0/91	0/001
96	68/82^{a}	65/93^{a}	67/61^{b}	56/12^{c}	1/23	0/001

a, b, c, d Means significant differences within the row ($p<0.05$)

on gas production. The results of the experiments were different based on each essential oil, doses, incubation time and feed substrate.

DISCUSSION

In the background of continuous the rumen flow, a change in development rate result changing in the rumen bacterial populations portion, also result changing in the fermentation profile (Griffin *et al*. 1999; Davidson and Naidu, 2000; Dorman and Deans, 2000; Cox *et al*. 2001). These actions could be more advantageous against gram-positive bacteria, in which the cell membrane can communicate directly with hydrophobic ingredients of EOs (Smith-Palmer *et al*. 1998; Chao and Young, 2000; Cimanga *et al*. 2002).

In this study, essential oils reclined gas production of all feed samples. These results of the findings are in agreement with the Cardozo et al. findings (2004). reduced *in vitro* gas production by essential oils show more effective energy utilizing because of the waste of energy as well as methane. Effects of EOs on rumen fermentation were significantly different. *Ziziphora clinopodioides* and *Mentha pulegium* against a wide range of gram-positive bacteria and gram-negative bacteria had bactericidal effects. Both are found in ORE (origanum essential oils) (Sivropoulou *et al*. 1996). Castillejos *et al*. (2006) presented that small amount of oregano (less than 50mg/l) didn't effect on microbial fermentation. however, a higher amount of *thymol* or oregano decreased total VFA (Castillejos *et al*. 2006) and reduced gas production (Akkan *et al*. 2006; Benchaar *et al*. 2007; Kamalak *et al*. 2011). Moreover, some researches suggested that oregano has an effect on diet and pH-dependent (Cardozo *et al*. 2005; Castillejos *et al*. 2006).

Although mainly emphasized on the antibacterial properties of essential oils, this is not their only effect. Gustafson and Bowen (1997) also represented that among other effects of essential oils, it's known they are capable to coagulation of some of the cellular components through the denaturation of the proteins. In addition, many types of research had revealed the capability of some phenolic and non-phenolic ingredients of essential oils to act reciprocally with chemical groups in protein structure and other active molecules, such as enzymes (Juven *et al*. 1994). Generally speaking, phenolic compounds communicate with protein ingredients via hydrogen bonds and electrostatic or hydrophobic bridges (Prescott *et al*. 2004), while non-phenolic ingredients communicate via other functional groups including the carbonyl group of cinnamaldehyde (Ouattara *et al*. 1997). In this study, silages treated with essential oils had higher protein content than control silage. Busquet *et al*. (2005) stated as the essential oil dose increased, the gas production was reduced. These results are in consistent with the present study.

Oh *et al.* (1967, 1968) reported that the low palatability of some plants to ruminants could be for both organoleptic effects, and their negative

Table 5. Gas production in different time of incubation in silages treated with Mentha pulegium

Time	Treatment (mg of Mentha pulegium) Control	10	20	30	SEM	P.value
2	22/76^{a}	20^{ab}	20/68^{ab}	19/2^{b}	0/93	0/09
4	31/87^{a}	26/71^{ab}	23/7^{b}	22/17^{c}	1	0/001
6	37/38^{a}	31/96^{b}	28/76^{c}	26/38^{c}	0/98	0/001
8	43/03^{a}	37/78^{b}	32/23^{c}	31^{c}	0/81	0/001
12	48/22^{a}	42/77^{b}	36/08^{c}	34/8^{c}	0/71	0/001
24	56/40^{a}	46/66^{b}	41/28^{c}	37/68^{d}	0/76	0/001
48	61/16^{a}	50/88^{b}	45/57^{c}	42/21^{d}	0/89	0/001
72	64/02^{a}	55/48^{b}	48/89^{c}	45/87^{d}	0/63	0/001
96	68/82^{a}	60/89^{b}	54/08^{c}	49/74^{d}	0/83	0/001

a, b, c, d Means significant differences within the row ($p<0.05$).

impact on rumen microbial fermentation, and nutrient digestion. In another study, Oh *et al*. (1967) examined the antibacterial activity of the EOs of *Pseudotsuga menziesii* and related ingredients on 24-h *in vitro* bath cultures by using of the ruminal fluid of deer and sheep. Their findings showed that doses of injection (4 to 8mL/L of fluid) were low and had not any positive impact on rumen fermentation performance, although higher doses (12mL/L of liquor) decreased gas yield during incubation time.

When the fundamental compounds isolated from the EO was utilized in same levels (3mL/L of liquor), those circular hydrocarbons (limonene and pinene) didn't change whether seldom motivated microbial activity, yet the cyclic hydrocarbons enrich with oxygen and particular alcohols (as terpinene and α-terpineol) hindered microbial activity in the rumen. It is famous that there is a strong link between gas production in laboratory studies and other *in vivo* experiments on rumen fermentation and microbial activity (Menke *et al*. 1979). The outcome of their findings indicated that essential oils could have a positive impact on the rumen microbial fermentation and nutrient digestion.

This is reported by benchaar *et al.* (2007) that gas production of *carvacrol, thymol*, and *eugenol* treatments reduced in comparison with control. These outcomes of their findings are not in agreement with the findings of oregano in the present experiment. Oregano possesses more *carvacrol* and eugenol compounds respectively than other essential oils. Therefore, garlic and oregano reduced gas production in barley on *in vitro* test. These results of the findings indicated that essential oils can be used to enhance digestion of leisurely starch degradation and monitor rate of releasing rapidly degradable starch to keep ruminal pH in the physiological range in the rumen.

Recently, the researchers have studied the impacts of active components of EOs on the performance of rumen microbial population. It is worth noting that the first challenge is to specify and determine which potential impacts are studied, and this may be varied with respect to the ration, cows, and production stage. Nevertheless, it is rational, to begin with recognizing and determining additives which develop propionic acid production and decline acetic acid and methane yields without any diminishing effect on VFA production. Also, the EOs which decrease microorganism activities such peptidolysis, proteolysis, deamination, and their association.

A series of *in vitro* short-term batch culture researchers have been utilized for monitor of capability helpful EOs (Cardozo et al. 2005; Busquet *et al*. 2006; Castillejos *et al.* 2006), and chosen essential oils and their important ingredients have been studied in long-term rumen fermentation researches (Cardozo et al. 2004; Busquet et al. 2005a, b, c; Castillejos *et al*. 2006).

The outcome of findings showed that essential oil of *garlic* and *cinnamaldehyde*, and *eugenol* that is the active element of the clove bud, and also *capsaicin* (important element of pepper), and *anethol* (main element of anise oil) enhance the profile of fermentation in continuous culture bath of microorganisms are located in rumen, and they Have been investigated in several *in vitro*, and some cases of *in vivo* studies (Cardozo *et al.* 2006).

Gas production on *In vitro* tests was reduced positively with essential oils. Most of the essential oils can be utilized to increase cellulose digestion and it can be regarded as a feed additive. One of the most important elements of *Mentha pulegium* and *Ziziphora clinopodioides* (up to79.33% in *Ziziphora clinopodioides*) is pulegone. In this present study, the pulegone content in essential oils is in consistent with the outcomes of Davidson and Naidu study (2000), that proposed, by using optimal doses, the efficiency of nutrient compound utilization in the rumen would be enhanced. Also, eugenol could increase production of VFA, and utilization of N in lactating animals rumen (Castillejos *et al.* 2006).

In a commercial form of essential oils, their main ingredients are thymol, eugenol, vanillin, carvacrol, and limonene, which can alter rumen fermentation. (McIntosh et al. 2003; Benchaar et al. 2007). the present experiment, CUM indicated the greatest gas production in comparison to control treatment at each test. ORE (origanum essential oils) also demonstrated the lowest gas production in all *in vitro* tests. In addition, observed essential Interactions related to the type of feeds, the duration of incubation and the dose that contradicts with findings of McIntosh *et al*. (2003). In this respect, Benchaar *et al*. (2007) claimed that observed variations and manipulation in the fermentation

of rumen created by EOs ingredients e.g carvacrol, eugenol and thymol may not be useful in lactation cows.

And also proposed that type and amount of essential oils and related ingredients should be meticulously specified and determined. In recent years, considerable knowledge has been achieved on the capability use of EOs to change microbial activity in the rumen. Although, before specifying recommendations for commercial use, several problems need to be addressed to be established. Most of these limitations of the present knowledge required to be resolved. For instance, the number of active ingredients in EOs Extensively depend on the variety, developing situations, and also technical method of extraction.

CONCLUSIONS

According to the present study, it can be found that estimated EOs and their compounds have a positive effect on rumen degradation due to relying on the essential oils and feeds applied. Although *in vitro* studies is still required to monitoring and checking new findings, then specifying functions of behavior, and also an important requirement to perform *in vivo* research so as to specify and specify the optimum doses in active element unit, the adaptation ability of rumen microorganisms to the action of this essential oils, the destiny of these additives in the gastrointestinal trace and the existence of residues in some products as milk and meat, and the impacts on performance of dairy cattle.

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