# EFFECT OF LAVENDER AND PEPPERMINT ESSENTIAL OIL ON *IN VITRO* METHANOGENESIS AND FERMENTATION OF FEED WITH BUFFALO RUMEN LIQUOR

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## ABSTRACT

The study was carried out to determine the effect of lavender (*Lavandula angustifolia*) and peppermint (*Mentha piperita*) essential oils (EO) (0, 1.5 and 3.0 µl/ml) on fermentation, digestibility, gas and methane production of feed with Buffalo rumen liquor. The study was performed with alfalfa and concentrate mixture (60:40) as a substrate and two buffalo was used as inoculum for rumen fluid in *in vitro* gas production. As a result, gas production was increased with supplementation of 1.5 µl/ml lavender and peppermint EO oil but decreased supplementation of 3.0 µl/ml ($P<0.0$) at 24 h after incubation. The methane production was decreased with lavender EO ($P<0.01$) as decreased by 45.57, 23.59 and 16.54 mL/g at 0, 1.5 and 3.0 µl/ml of lavender EO, respectively. There was a decrease in *in vitro* methane production (%) with high levels of peppermint oil ($P<0.05$) but gas production was decreased with a high dose (3.0 µl/ml). In the present study *in vitro* true dry matter digestibility (TDMD) and organic matter digestibility decreased with high doses supplementation of lavender and peppermint oil ($P<0.01$). Lavender EO had no effect on rumen volatile fatty acids production and pH ($P>0.05$). Rumen pH was not affected with lavender and peppermint EO supplementation ($P>0.05$), that changed within normal range of rumen pH (6.5 to 7.0). Ruminal acetate production was increased, and propionate and butyrate production were decreased with 3.0 µl/ml peppermint EO supplementation. Lavender EO was more effective for reduction of methane production without negative effects (especially gas production and TDMD) but this effect becomes negative effects at higher doses of EO supplementations. So, lavender and peppermint EO could be need more study as a feed additive to reduce methanogenesis in *in vivo* experiments.

**Keywords**: *Bubalus bubalis*, buffaloes, methanogenesis, *Lavandula angustifolia*, *Mentha piperita*, rumen fermentation

## INTRODUCTION

In recent years, the global warming caused by the greenhouse effect is due to the accumulation in the atmosphere of sera gases. These gases are mainly carbon dioxide, methane, chlorofluorocarbons and nitrogen oxides. Methane gases plays a huge role in global warming because of its high greenhouse gas potential (about 23 × $CO_2$, IPCC, 2006), that is

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a by-product of anaerobic microbial fermentation of carbohydrates in the rumen. So, it is produced in considerable amounts of ruminants (Janssen, 2010). In addition to causing global warming, it causes an energy loss to the animals between 2 and 15% (Flachowsky and Brade, 2007). Therefore, it is crucial to reduce enteric methanogenesis for environment protection and increase the performance in ruminants. However, it should be noted that there are natural products to reduce ruminal methanogenesis. Also, there are greatly limitations about feed additives for consumer protection in European legislation. Therefore, alternative new feed additives are primordial. Recently, the plant secondary metabolites which are natural products using as feed or food additive seems to be a better way to reduce environmental negative effects and it's a better choice for the consumers. Plant essential oils (EO) are identified that secondary metabolites and which selectively inhibit rumen methanogenesis without depressing feed intake and digestion, as well as animal health and productivity in *in vivo* studies (Flachowsky and Lebzien, 2012). Also, EOs are known as have anti-methanogenic and antiprotozoal activity (Patra *et al*., 2006; Kamalak *et al*., 2011). In previous study, peppermint (*Mentha piperita*) and lavender (*Lavandula angustifolia*) essential oils have anti-microbial activity and are commonly used for the treatment of microbial infections (Imai *et al*., 2001; Hanamanthagouda *et al*., 2010) and are used for anti-methanogenic (Agarwal *et al*., 2009). The results of EO's anti-methanogenic and antimicrobial effects presented in previous studies but the mechanism and mode od action needs to be detailed with different doses. In previously, the studies showed that EO supplementation reduced methane production linearly, but the toxic levels not known exactly. Therefore, the optimum dose of EO supplementation need to be optimized without affecting feed digestibility and animal performance. Studies have shown that essential oils reduce *in vitro* methanogenesis due to the dosage that methane production decreases as the dose increases (Agarwal *et al*., 2009). Therefore, the supplementation dose of EOs to diet is very important for better use of these products to be used as additives.

The present experiment has been conducted that the anti-methanogenic potential of lavender (*Lavandula angustifolia*) and peppermint oil (*Mentha piperita*) with high dosage in Buffalo rumen liquor.

## MATERIALS AND METHODS

### Experimental design

*Lavandula angustifolia* and *Mentha piperita* essential oil (the major constituents were linalyl acetate for *L. angustifolia* and menthol for *M. piperita*) extracted, were tested at three levels (0, 1.5 and 3.0 µl/ml) with 200 mg feed substrate in 100 ml graduated syringes and with five replicates for each treatment. In incubation set of syringes comprised of five syringes for each treatment (oil supplemented) and ten control (five syringes for each treatment) without any supplementation, three syringes as standard (alfalfa hay as substrate) and two syringes as blanks (without substrate).

### Preparation of inoculum

Rumen liquor was collected from two buffaloes fed on a diet of alfalfa and concentrate mixture in 60:40 ratio according to Chaudhry (2006) at slaughter. The alfalfa had dry matter (DM), 919.7 g; organic matter (OM), 887.1 g; crude protein (CP), 163.6 g; neutral detergent fibre (NDF),

349.3 g and acid detergent fibre (ADF), 267.7 g/kg and the concentrate mixture (consisting of wheat bran, 24; maize, 22; barley, 10; maize bran, 12; soybean meal, 18; palm meal; 2; soybean bran, 9; vitamin-mineral premix, 2 and salt, 1 kg/100 kg) had DM, 906.6 g; OM, 968.1 g; CP, 143.3 g; crude cellulose (CC), 93.4 g/kg on DM basis. Ruminal fluid was collected immediately post-mortem from the tree different site of rumen and filtered through four layers of cheese cloth and used as a source of inoculum. The ruminal contents were bubbled with $CO_2$ and maintained at 39°C during the incubation process. The addition and dosage of EO extract in syringes were performed at the time of incubation.

***In vitro* gas production test**

Alfalfa and concentrate mixture (60:40 ratio) were used as *in vitro* test substrate (milled to pass through 1 mm sieve and was weighed 200±10 mg in glass syringes of 100 ml capacity was. The incubation was prepared according to the method defined by Menke and Steingass (1988). The medium (10 ml of rumen fluid + 20 ml buffer solution) was dispensed automatically and anaerobically in each syringe. The syringes were pre-heated and were transferred to a special water bath previously heated to 39°C for 24 h incubation. The syringes were gently shaken every 30 minutes during the first 12 h of incubation. After 3, 6, 12 and 24 h of incubation, bottles were taken to determine the gas production. The pH, gas and methane production analysis were performed after 24 h incubation. pH measurements were made using a pH meter (WTW, Weilheim, Germany). After pH measurements all fluid samples were sampled (about 4 ml) for methane and volatile fatty acid (VFA) analysis.

**Determination of gas and methane production**

Gas production was determined by the displacement of piston in the syringe at the end of the 24 h of incubation. For the net gas production, the gas produced from the treatments was calculated by determining the difference from the gas produced in the empty syringe (no substrate contain, contains medium). In each incubation, 5 empty syringes were used, and the gas produced was calculated by averaging the gas produced in these 5 syringes. In addition, standard (containing standard alfalfa hay) was used as the substrate in order to eliminate differences between incubations and changes in ruminal fluid. Gas production (GP) results are presented as ml/g (GP for 200 mg × 5).

After measuring the total net gas volume in 24 h, the gas produced in the syringe was withdrawn from the outlet of the syringe with the aid of a plastic syringe and placed at the inlet of the methane analyzer (Sensors Europe GmbH, Erkrath, Germany). The plunger was pushed to introduce the gas accumulated in the syringe into the analyzer and the methane ratio of total gas was shown in % on a computer (Goel *et al*., 2008).

**Volatile fatty acid analysis**

For volatile fatty acid analysis, 24 h after incubation, the supernatant (about 1 ml) was collected in a tube then, added 0.20 ml metaphosphoric acid (25%) and centrifuged at 5000 × g for 10 min after standing 2 h at room temperature. The supernatant was collected from clear phase with a pipette and stored at −20°C until the analyses. The VFA concentrations (acetic, propionic and butyric acids, %) were analyzed on a gas chromatography (Schimadzu GC 2010 Plus, Japan) as described by Erwin *et al.* (1961). Gas chromatography was equipped with a flame ionization detector (FID) and stabilwax capillary

column (30 m, 0.25 mm ID, 0.25 μm). Into the device gas flow rates for nitrogen, hydrogen and air were 30, 30 and 320 ml/minutes, respectively. Injector, column, and detector's temperature were 270, 172 and 270°C, respectively.

***In vitro* digestibility**

*In vitro* true dry matter digestibility (TDMD) was determined by the DaisyII Incubation method. The rumen fluid obtained from used in the gas production analysis. The substrates were weighted bags and transferred to a pre-heated digestion jar (containing 400 ml rumen fluid + 1600 ml buffer solution (pH 6.8)). The bags were incubated in five replicates at 39.5°C for 48 h. At the end of the incubation, the bags were washed with cold water and dried after followed by NDF digestion in an Ankom200/220 Fiber Analyzer (Ankom, Fairport, NY, USA). Organic matter digestibility (OMD, %) = 14.88+0.8893GP+ 0.448CP+0.651Ash where GP is production (24 h, ml/200 mg DM); CP: crude protein and Ash in percent DM.

**Essential oil composition of plants**

EO extraction was made by the hydro-distillation process. For EO distillation 100 g of plant material (lavender and peppermint) were introduced in 500 ml of distilled water. The water was boiled with heated mantles, and the condensed EO was deposited and separated with the aid of the cooling system. The EO obtained was stored in dark bottles at +4°C until use in incubation. Essential oil composition analysis for each EO was analyzed in a GC-MS and were determined following the procedure described by Beyzi *et al*. (2017).

**Proximate analyses**

The DM, CP (N×6.25), OM and crude ash of substrates were analyzed according to AOAC (1995) procedures. The ADF, NDF and CC were analyzed according to the methods described by Van Soest *et al*. (1991).

**Statistical analyses**

The data were analyzed using one-way ANOVA procedure of SPSS (1996) and determining the differences between the means were compared using Duncan's new multiple range test.

## RESULTS AND DISCUSSION

**Effects of lavender oil**

The effect of lavender essential oil on *in vitro* gas production, methane production, pH, volatile fatty acids (VFA) production and digestibility are represented in Table 1. Gas production was increased with supplementation of 1.5 μl/ml lavender essential oil but decreased supplementation of 3.0 μl/ml lavender essential oil ($P<0.01$). There are limited data in the literature about lavender EO, in a study lavender EO had a stimulatory effect on *in vitro* gas production (Broudiscou and Lassalas, 2000); however, the stimulatory effect of lavender EO was related to doses. While lavender EO increases gas production at low dose, it is determined that gas production decreases at high levels. Similar to our results in another experiment, supplementing with lavender EO (up to 500 ppm) resulted a decrease in gas production with increasing doses of lavender EO (up to 1000 ppm) (Yadeghari *et al*., 2015). In present study the methane production was decreased with lavender EO ($P<0.01$) supplementation. Lavender EO has a depressive effect on *in vitro* rumen methanogenesis. EOs possible anti-

methanogenesis effects 1) directly inhibiting the methanogens (Ohene-Adjei *et al*., 2008) 2) indirectly by their negative impact on ciliate protozoa or as a consequence of reduced organic matter degradation, providing the precursors for methane production (Patra *et al*., 2006; Jouany and Morgavi, 2007). Tekippe *et al.* (2012) examined that lavender (*Lavandula latifolia*) essential oil reduced methane production by 20 to 30% in cattle under *in vitro* conditions. Yadeghari *et al*. (2013) showed that lavender EO had an inhibitory effect on ruminal *in vitro* methanogenesis with ewe rumen fluid. In present study for the higher gas production despite a decrease in TDMD and OMD as lavender EO dosage increased. Also, lavender EO had no effect on rumen VFA production and pH (P>0.05). However, Broudiscou *et al.* (2002) showed that a stimulatory effect of lavender EO on VFA production. In another experiment, lavender EO had no effect on rumen fermentation at the doses up to 500 mg/L (Castillejos *et al*., 2008). These results show that increasing in gas production does not appear to be a result of higher organic matter digestibility. Rumen liquor pH was not affected with lavender EO supplementation (P>0.05), that changed within normal range of rumen pH (6.5 to 7.0) which indicating no adverse effect of EO addition on rumen environmental conditions. In the study, although methane production decreased and gas production increased with 1.5 µl/ml lavender EO, acetate production did not show a significant increase. This shows that a part of gas production during ruminal fermentation is due to VFA buffering (Blummel and Orskov, 1993). Therefore, lavender EO was used the carbon in rumen by microorganisms, that resulted as high amount of gas production. But same mechanism was not available for 3.0 µl/ml lavender EO supplementation that this dose is also considered to be toxic to rumen microorganisms. Also, it may be related to lower TDMD at this dose of lavender EO.

**Effects of peppermint EO**

The effect of peppermint essential oil on *in vitro* gas production, methane production, pH, volatile fatty acids (VFA) production and digestibility are represented in Table 2. Gas production was increased with supplementation of 1.5 µl/ml peppermint essential oil however decreased supplementation of 3.0 µl/ml peppermint essential oil (P<0.01). Ozkan *et al*. (2015) showed that supplementation of peppermint essential oil (0 to 1200 mg/L) decreased *in vitro* gas production. Similar findings were presented by Canbolat *et al*. (2011); Canbolat (2012). In present study results were similar with Agarwal *et al*. (2009) that the gas production increased with 0.33 and 1.0 µl/ml of peppermint oil, but at 2.0 µl/ml level it was similar to that in control. *In vitro* methane production (%) was decreased with high level (Table 2) however gas production was also decreased with high dose of peppermint oil. In previous study, there was a decrease in methane production with increasing peppermint oil doses (at 0 to 2 µl/ml) (Agarwal *et al*., 2006; 2009). In present study, TDMD and OMD were decreased with peppermint oil supplementation at 3.0 µl/ml level (P<0.01). Similar results were showed by Singh *et al*. (2018) with lemongrass EO supplementation in buffalo. The possible mechanism described in the study with lavender oil also applies to peppermint oil. Also, the increasing in DM digestibility with EO supplementation could be due to inhibition of responsible for the degrading of cellulose bacteria. Rumen liquor pH was not affected with peppermint EO supplementation (P>0.05), that changed within normal range of rumen pH (6.5 to 7.0) which

indicating no adverse effect of EO addition on rumen environmental conditions. Effects of peppermint EO on ruminal VFA productions are presented in Table 2. Ruminal acetate production was increased, and propionate and butyrate production were decreased with 3.0 µl/ml peppermint EO supplementation. Related to acetate increment ruminal A/P which might be due to accumulation of molecular hydrogen was increased in 3.0 µl/ml peppermint EO supplementation. These results are in agreement with results of Ozkan *et al*. (2015); Canbolat *et al*. (2011); Canbolat (2012) suggested that peppermint EO decreased propionate and butyrate and increased the acetate and A/P ratio. It is known that increasing of propionate production and A/P ratio in rumen are caused by a decrease in methane production (Russell, 1998). In this study, methane production decreased by 3.0 µl/ml peppermint EO, but gas production also decreased significantly. Similarly, Agarwal *et al*. (2009) the peppermint EO was increased the acetate and A/P ratio with linearly (0 to 2 µl/ml).

**Effects of lavender and peppermint EO**

The effects of lavender and peppermint EO on DM digestibility, gas production and methane production are showed in Figure 1 and 2. The gas production (ml/g) increased with 1.5 µl/ml peppermint and lavender EO, but at 3.0 µl/ml level it was decreased that in control ($P<0.05$). The TDMD was ranged with 65.1 to 76.3 % that it was similar in control, 1.5 µl/ml lavender and peppermint EO groups, however it was decreased that in peppermint EO at 3.0 µl/ml level. Methane production values significantly decreased in time in all treatment groups for all feeds ($P<0.05$). The lowest methane production (ml/g) was observed in peppermint EO with 3.0 µl/ml, but the highest production was in control and 1.5 µl/ml peppermint EO. The methane production (%) values for control and 1.5 and 3.0 µl/ml lavender and peppermint EO supplemented groups at 24 h were respectively 16.7, 7.6, 12.1, 14.5 and 8.0% while the lowest methane production value (7.6) was recorded in the 1.5 µl/ml lavender EO supplementation ($P<0.05$).

## CONCLUSION

In the study, the lavender and peppermint EO have similar effects on gas and methane production and TDMD but, differently affected the ruminal fermentation that lavender EO has no effect but peppermint EO has a stimulatory effect on ruminal fermentation which acetate and A/P ratio. The present study showed that a stimulatory effect of lavender and peppermint EO at low dose on rumen gas production and reducing methane production, however high dose has negative effects on gas production and digestibility. In conclusion, lavender and peppermint EO have a potential to reduce methanogenesis, ruminal fermentation and improve animal performance. As indicated by the findings of the study, lavender EO was more effective for reduction of methane production without negative effects (especially gas production and TDMD) but this effect becomes negative effects at higher doses of EO supplementations. However, effects of EOs on methanogenesis, rumen fermentation and feed digestibility may vary according to their dose, feed substrate and amount of bio-active metabolites of EOs. Regarding the positive effect of lavender and peppermint EO to modify gas and methane production, ruminal fermentation parameters it can be used as feed additive at the doses up to 3.0 µl/ml.

Table 1. Effect of lavender essential oil supplementation on *in vitro* gas and methane production, feed digestibility, pH and fermentation parameters in 24 h.

| Attributes | Lavender essential oil, µl/ml | | | SEM | P-value |
|---|---|---|---|---|---|
| | 0 | 1.5 | 3.0 | | |
| Gas production, ml/g DM | 266.3[b] | 312.5[a] | 136.7[c] | 14.82 | <0.001 |
| Methane production, ml/g | 45.57[a] | 23.59[b] | 16.54[b] | 4.22 | <0.001 |
| Methane production, % | 17.10[a] | 7.55[c] | 12.10[b] | 1.10 | 0.001 |
| TDMD | 76.25[a] | 76.37[a] | 68.51[b] | 1.21 | <0.001 |
| OMD | 77.51[b] | 85.73[a] | 56.41[c] | 1.98 | <0.001 |
| pH | 6.82 | 6.85 | 6.83 | 0.04 | 0.741 |
| Acetate, % | 64.33 | 65.15 | 64.54 | 0.52 | 0.078 |
| Propionate, % | 28.08 | 27.36 | 28.16 | 0.24 | 0.114 |
| Butyrate, % | 7.58 | 7.49 | 7.30 | 0.25 | 0.158 |
| A/P | 2.30 | 2.38 | 2.29 | 0.04 | 0.180 |

SEM: standard error of means; P: probability;

TDMD: true dry matter digestibility; OMD: organic matter digestibility.

[a,b,c]: means with different superscripts within a row differ significantly.

Table 2. Effect of peppermint essential oil supplementation on *in vitro* gas and methane production, feed digestibility, pH and fermentation parameters in 24 h.

| Attributes | Peppermint essential oil, µl/ml | | | SEM | P-value |
|---|---|---|---|---|---|
| | 0 | 1.5 | 3.0 | | |
| Gas production | 266.7[b] | 315.0[a] | 105.0[c] | 15.03 | <0.001 |
| Methane production, ml/g | 42.89[a] | 45.51[a] | 8.35[b] | 3.99 | <0.001 |
| Methane production, % | 16.07[a] | 14.45[a] | 7.95[b] | 1.06 | 0.001 |
| TDMD | 76.27[a] | 76.33[a] | 65.13[b] | 1.07 | <0.001 |
| OMD | 77.59[b] | 86.19[a] | 51.15[c] | 1.58 | <0.001 |
| pH | 6.82 | 6.75 | 6.68 | 0.04 | 0.681 |
| Acetate, % | 64.33[b] | 65.93[b] | 70.05[a] | 0.60 | 0.001 |
| Propionate, % | 28.08[a] | 28.27[a] | 26.10[b] | 0.30 | 0.044 |
| Butyrate, % | 7.58[a] | 5.80[b] | 3.85[c] | 0.38 | <0.001 |
| A/P | 2.30[b] | 2.34[b] | 2.70[a] | 0.05 | 0.017 |

SEM: standard error of means; P: probability; TDMD: true dry matter digestibility;

OMD: organic matter digestibility.

[a,b,c]: means with different superscripts within a row differ significantly.

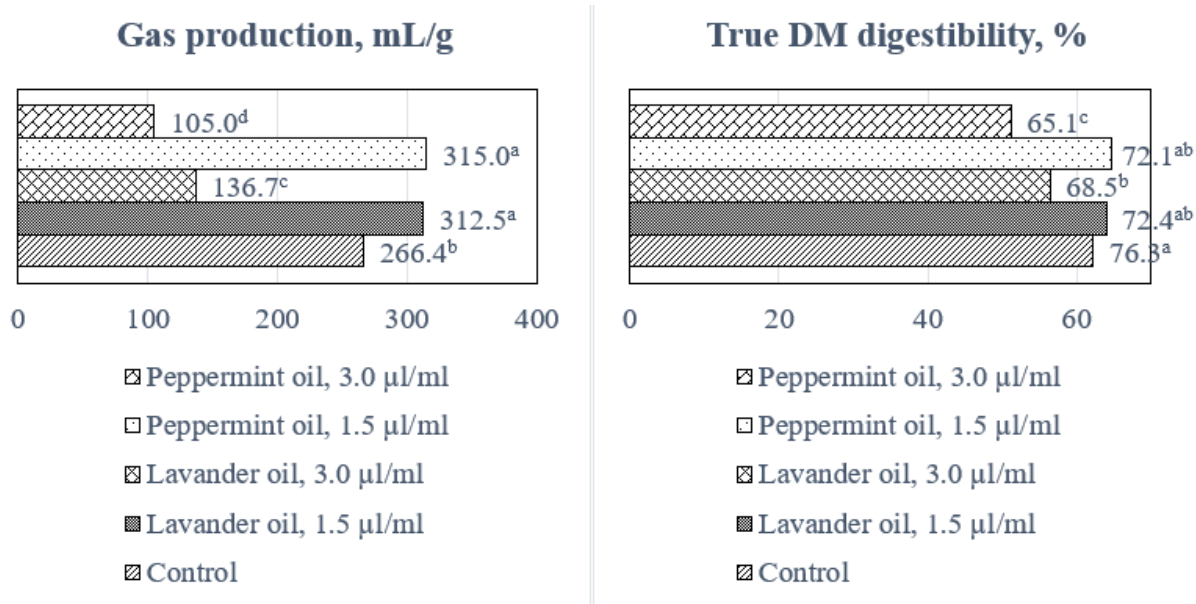


Figure 1. *In vitro* DM digestibility and gas production supplemented with lavender and peppermint essential oils.

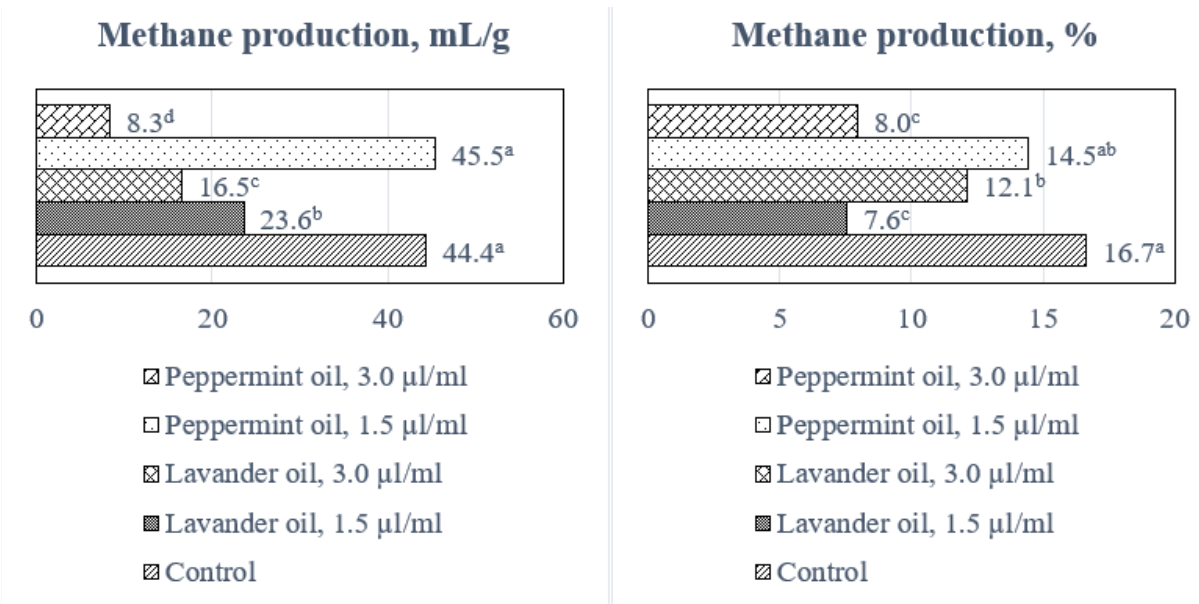


Figure 2. *In vitro* ruminal methanogenesis supplemented with lavender and peppermint essential oils.

## REFERENCES


Agarwal, N., D.N. Kamra, L.C. Chaudhary and A.K. Patra. 2006. Effect of *Sapindus mukorossi* extracts on *in vitro* methanogenesis and fermentation characteristics in buffalo rumen liquor. *J. Appl. Anim. Res*., **30**(1): 1-4. DOI: 10.1080/09712119.2006.9706814

Agarwal, N., C. Shekhar, R. Kumar, L.C. Chaudhary and D.N. Kamra. 2009. Effect of peppermint (*Mentha piperita*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. *Anim. Feed Sci. Tech*., **148**(2): 321-327. DOI: 10.1016/j.anifeedsci.2008.04.004

AOAC. 1995. *Official Methods of Analysis*, 16th ed. Association of Official Analytical Chemists, Washington DC, USA.

Beyzi, E., K. Karaman, A. Gunes and S.B. Beyzi. 2017. Change in some biochemical and bioactive properties and essential oil composition of coriander seed (*Coriandrum sativum* L.) varieties from Turkey. *Ind. Crop. Prod*., **109**(15): 74-78. DOI: 10.1016/j.indcrop.2017.08.008

Blummel, M. and E.R. Orskov. 1993. Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Anim. Feed Sci. Tech*., **40**(2-3):109-119. DOI: 10.1016/0377-8401(93)90150-I

Broudiscou, L.P., Y. Papon and A.F. Broudiscou. 2002. Effects of dry plant extracts on feed degradation and the production of rumen microbial biomass in a dual outflow fermenter. *Anim. Feed Sci. Tech*., **101**(1-4): 183-189. DOI: 10.1016/S0377-8401(02)00221-3

Broudiscou, L.P. and B. Lassalas. 2000. Effects of Lavandula officinalis and *Equisetum arvense* dry extracts and isoquercitrin on the fermentation of diets varying in forage contents by rumen microorganisms in batch culture. *Reprod. Nutr. Dev.*, **40**(5): 431-440. DOI: 10.1051/rnd:2000110

Canbolat, O., H. Kalkan, S. Karaman and I. Filya. 2011. The effect of essential oils on the digestibility, rumen fermentation and microbial protein production. *Kafkas Univ. Vet. Fak*., **17**(4): 557-565. DOI: 10.9775/kvfd.2011.4006

Canbolat, O. 2012. The effect of some essential oils on *in vitro* digestibility, rumen fermentation characteristics and methane gas production. *Igdir University Journal of the Institute of Science and Technology*, **2**: 91-98.

Castillejos, L., S. Calsamiglia, J. Martin-Tereso and H.T. Wijlen. 2008. *In vitro* evaluation of effects of ten essential oils at three doses on ruminal fermentation of high concentrate feedlot-type diets. *Anim. Feed Sci. Tech*., **145**(1-4): 259-270. DOI: 10.1016/j.anifeedsci.2007.05.037

Chaudhry, A.S. 2006. *In vitro* degradation of forages by using rumen fluid from slaughtered cattle, p. 17-20. *In Proceedings of 57th Annual Meeting of the EAAP*, Antalya, Turkey.

Erwin, E.S., G.J. Marco and E.M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J. Dairy Sci*., **44**(9): 1768-1771. DOI: 10.3168/jds.S0022-0302(61)89956-6

Flachowsky, G. and W. Brade. 2007. Potenziale zur Reduzierung der methan-emissionen bei Wiederkäuern. *Züchtungskunde*, **79**(6): 417-465.

Flachowsky, G. and P. Lebzien. 2012. Effects

of phytogenic substances on rumen fermentation and methane emissions: A proposal for a research process. *Anim. Feed Sci. Tech*., **176**(1-4): 70-77. DOI: 10.1016/j.anifeedsci.2012.07.009

Goel, G., H.P. Makkar and K. Becker. 2008. Effects of *Sesbania sesban* and *Carduus pycnocephalus* leaves and Fenugreek (*Trigonella foenum-graecum* L.) seeds and their extracts on partitioning of nutrients from roughage-and concentrate-based feeds to methane. *Anim. Feed Sci. Tech*., **147**(1-3): 72-89. DOI: 10.1016/j.anifeedsci.2007.09.010

Hanamanthagouda, M.S., S.B. Kakkalameli, P.M. Naik, P. Nagella, H.R. Seetharamareddy and H.N. Murthy. 2010. Essential oils of *Lavandula bipinnata* and their antimicrobial activities. *Food Chem*., **118**(3): 836-839. DOI: 10.1016/j.foodchem.2009.05.032

Imai, H., K. Osawa, H. Yasuda, H. Hamashima, T. Arai and M. Sasatsu. 2001. Inhibition by the essential oils ofpeppermint and spearmint of the growth of pathogenic bacteria. *Microbios*, **106**(Suppl. 1): 31-39.

IPCC. 2006. 2006. *IPCC Guidelines for National Greenhouse Gas Inventories*. National Greenhouse Gas Inventories Program, Intergovernmental Panel on Climate Change, Geneva, Switzerland.

Janssen, P.H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Anim. Feed Sci. Tech*., **160**(1-2): 1-22. DOI: 10.1016/j.anifeedsci.2010.07.002

Jouany, J.P. and D.P. Morgavi. 2007. Use of 'natural'products as alternatives to antibiotic feed additives in ruminant production. *Animal*, **1**(10): 1443-1466. DOI: 10.1017/S1751731107000742

Kamalak, A., Ö. Canbolat, Ç.Ö. Özkan and A. Atalay. 2011. Effect of thymol on *in vitro* gas production, digestibility and metabolizable energy content of alfalfa hay. *Kafkas Univ. Vet. Fak.*, **17**: 211-216. DOI: 10.9775/kvfd.2010.2854

Menke, K.H. and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Animal Research and Development*, **28**: 7-55.

Ohene-Adjei, S., A.V. Chaves, T.A. McAllister, C. Benchaar, R.M. Teather and R.J. Forster. 2008. Evidence of increased diversity of methanogenic archaea with plant extract supplementation. *Microb. Ecol*., **56**(2): 234-242. DOI: 10.1007/s00248-007-9340-0

Ozkan, C.O., A. Kamalak, A.I. Atalay, A. Tatliyer and E. Kaya. 2015. Effect of peppermint (*Mentha piperita*) essential oil on rumen microbial fermentation of barley grain. *J. Appl. Anim. Res*., **43**(3): 287-290. DOI: 10.1080/09712119.2014.963101

Patra, A.K., D.N. Kamra and N. Agarwal. 2006. Effect of plant extracts on *in vitro* methanogenesis, enzyme profile and fermentation of feed in rumen liquor of buffalo. *Anim. Feed Sci. Tech*., **128**(3-4): 276-291. DOI: 10.1016/j.anifeedsci.2005.11.001

Russell, J.B. 1998. The importance of pH in the regulation of ruminal acetate to propionate ratio and methane production *in vitro*. *J. Dairy Sci*., **81**(12): 3222-3230. DOI: 10.3168/jds.S0022-0302(98)75886-2

Singh, R.K., A. Dey, S.S. Paul, M. Singh and B.S. Punia. 2018. Responses of Lemongrass (*Cymbopogon citratus*) essential oils

supplementation on *in vitro* rumen fermentation parameters in buffalo. *Indian J. Anim. Nutr.*, **35**(2): 174-179. DOI: 10.5958/2231-6744.2018.00026.9

SPSS. 1996. *Statistical Packages for Social Sciences*, version 7.5. SPSS Inc., Illinois, USA.

Tekippe, J.A., A.N. Hristiov, K.S. Heyler, V.D. Zheljazkov, J.F.S. Ferreira, C.L. Cantrell and G.A. Varga. 2012. Effects of plants and essential oils on ruminal *in vitro* batch culture methane production and fermentation. *Can. J. Anim. Sci*., **92**: 395-408. Available on: https://www.nrcresearchpress.com/doi/pdf/10.4141/cjas2012-059

Van Soest, P.J., J.B. Robertson and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci*., **74**(10): 3583-3597. DOI: 10.3168/jds.S0022-0302(91)78551-2

Yadeghari, S., M. Malecky, M.D. Banadaky and B. Navidshad. 2015. Evaluating in vitro dose-response effects of *Lavandula officinalis* essential oil on rumen fermentation characteristics, methane production and ruminal acidosis. *Veterinary Research Forum*, **6**(4): 285-293.

Yadeghari, S., M. Malecky, P. Zamani and M. Dehghan-Banadaky. 2013. *In vitro* evaluation of the effects of *Lavandula officinalis* and *Origanum vulgare* essential oils on ruminal fermentation using concentrate and roughage type substrates. *Journal of Livestock Science and Technologies*, **1**(2): 1-12.