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The effect of *Origanum syriacum* L. extract and carvacrol on the *in vitro* digestion, estimated digestion values, ammonia and organic acid concentrations in the fermentation fluid of lucerne herbage

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Abstract: The effects induced by medicinal aromatic plants in biological systems vary with the type and amount of bioactive substances these plants contain. Whether the purified form of the main chemical components of these plants, such as carvacrol and thymol, or plant volatile oils containing tens of bioactive compounds are more effective remains a question of debate. This study was aimed at providing a comparative assessment of the effects of *Origanum syriacum* L. (wild mountain thyme) volatile oil (OSVO) and one of its main components, carvacrol (CRV), on the *in vitro* ruminal degradability of lucerne herbage and methane production during the degradation of lucerne. For this purpose, wild thyme was harvested at the beginning of the flowering period, and the OSVO was extracted from the plant by steam distillation. Gas production assays were performed in five groups of ruminal fluid samples, one of which was maintained for control purposes, and the other four 40/60/80 mg/l of OSVO and 60 mg/l of CRV were added. Compared to the control group, in the samples with the added CRV and OSVO, the amounts of *in vitro* total gas and methane production were observed to have been affected, but no decrease was detected in the ruminal protozoa counts. The level of ammonia nitrogen was lowest in the groups, in which CRV and 40 mg/l of OSVO ($P < 0.01$) were added. The ruminal protozoa counts were not affected by the addition of CRV and OSVO. While the total volatile fatty acid (TVFA) and propionic acid (PA) concentrations in the *in vitro* fermentation fluid of lucerne herbage were low in all the groups, butyric acid was detected at a level of 40 mg/l in the group where CRV was added. The OSVO was ascertained to have induced dose-dependent alterations in the investigated *in vitro* digestion parameters. In result, CRV (60 mg/l) and OSVO (40 mg/l) were determined to have shown a relatively positive effect on the *in vitro* ruminal gas production. The anti-methanogenic effect of the plant extracts was due to the decreased digestibility of the lucerne herbage. This can have a positive impact on the environment, but the same cannot be said for the animal nutrient use and animal performance.

Keywords: carvacrol; *in vitro* gas production; methane; thyme; volatile oil

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Today, global food safety is faced with multiple challenges, including, among others, the greenhouse effect, global warming and an increasing world population. Almost half of the global carbon dioxide (CO_2) and methane (CH_4) emissions have occurred in the past 40 years (IPCC 2014). Out of the 80 million tonnes of global annual CH_4 emissions, 47% originate from agricultural land activities and 39% originate from animal production, and these percentages are reported to increase by almost 50% in rural areas (Gerber et al. 2013; Jafari et al. 2019). Depending on the type, particle size and dry matter content of the feed provided to the animals, the amount of CH_4 produced per head is reported to range between 60–160 kg/year for cattle and 10–16 kg/year for sheep and goats (Hristov et al. 2013). In ruminants, methane production is reported to cause a 2–12% loss in the gross energy (Wanapat et al. 2015). No environmental hazard has been reported to be associated with the use of volatile oils (VOs) as feed additives, such that, when administered at the recommended doses, the use of VOs in animal nutrition is considered to be safe (Baytok et al. 2017; Onel and Aksu 2020).

VOs show an anti-methanogenic effect on the *in vitro* degradation and they alter the ruminal fluid parameters (Cobellis et al. 2016; Onel et al. 2020; Zhou et al. 2020). Carvacrol, a major active phenolic compound found in thyme, is responsible for the antibacterial effect of this plant. However, only limited information is available on the dose-dependent effect of carvacrol on the enteric CH_4 production (Soltan et al. 2011). Owing to the active substances found in its composition, thyme volatile oil has the potential of altering the ruminal fluid indicators and the *in vitro* degradation. Thus, the present study was designed to provide scientific data on the use of VOs and phenolic compounds as feed additives to reduce the enteric CH_4 production in ruminants, and thereby contributing to minimising the effects of global climate change and improving the ruminant production.

The Mediterranean flora includes several thyme species (*Thymbra spicata* L., *Origanum syriacum* L., *Origanum onites* L. and *Thymbra sintenisii* subsp. *isaurica*). Volatile oils extracted from these species contain the secondary compounds carvacrol and thymol, which determine the functional effects of the plant. The carvacrol content of thyme species is reported to show a wide variation (19.23–34.10%) (Onel et al. 2018). *In vitro* studies have demonstrated the anti-methanogenic and antibacterial effects of carvacrol during ruminal fermentation (Soltan et al. 2011; Castaneda-Correa et al. 2019). It is clear that the effect of OSVO on the ruminal fermentation and feed digestion is directly related to the active substances it contains. This study is based on the hypothesis that the effects of *Origanum syriacum* L. volatile oil, administered at different doses, and pure carvacrol on the *in vitro* ruminal degradation and methane production may differ. Thus, this study was designed to determine the effects of carvacrol alone and the combination of active substances found in *Origanum syriacum* L. (carvacrol + thymol + γ-terpinene + *p*-cymene). The aim of the present study was to present a comparative assessment of the effects of carvacrol (60 mg/l) and *Origanum syriacum* L. volatile oil (40/60/80 mg/l) on the *in vitro* ruminal gas production and methane emission associated with the degradation of lucerne herbage, as well as on the ruminal organic acid concentrations and protozoa counts.

MATERIAL AND METHODS

Ethical approval

We obtained an approval (No. 2020/05-2) from the Local Ethics Committee of Hatay Mustafa Kemal University for this study.

Plant material

The plant volatile oil used in this study was extracted by steam distillation from *Origanum syriacum* L. leaves, which were harvested during the flowering period and later dried at 35 °C.

Extraction and component analyses of *Origanum syriacum* L. volatile oil

The dried plant material was chopped and placed in a beaker, then steam distillation was used to extract the essential oil. Steam distillation is based on the principle of applying pressure to the plant materials using steam, creating droplets of oil and water together, then evaporating the water from the droplets in the beaker. The chemical components of the volatile oil were determined using

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an ISQ Single Quadrupole model gas chromatograph and a TG-Wax MS-A model (both Thermo Fisher Scientific, Waltham, MA, USA), a 5% phenyl polysilphenylene-siloxane column with a 0.25 mm inner diameter, 30 m in length, and a 0.25 µm film thickness. Helium (99.9%) was used as the carrier gas at a flow rate of 1 ml/minute. The ionisation energy was 70 eV and the mass range (m/z) was set from 1.2 amu to 1 200 amu. The scan mode was used for the data collection. The temperature of the mass spectrometry (MS) transfer line was 250 °C, the MS ionisation temperature was 220 °C, the temperature of the injection port was 220 °C, and the column temperature was initially 50 °C and increased up to 220 °C at a rate of 3 °C/minute. The structure of each component was described using the Xcalibur software (Thermo Fisher Scientific, USA) and mass spectra.

Chemical analysis of dried lucerne herbage

Lucerne herbage, which was harvested during the vegetation period and subsequently dried, was analysed for the dry matter (DM), crude ash (CA), crude protein (CP), and crude fat (CF) using the official analysis methods of the Association of Official Analytical Chemists (AOAC 1995). The composition of the neutral-detergent fibres (NDFs), acid-detergent fibres (ADFs) and acid detergent lignin (ADL) were analysed as described by Van Soest et al. (1991).

In vitro gas production

The *in vitro* degradability of the dried lucerne herbage was determined by the *in vitro* gas production assay described by Menke et al. (1979). Approximately one litre ruminal fluid samples were collected from (using a gastric tube) each of two Brown Swiss beef cattle, weighing 500–550 kg, and were transported to the laboratory in insulated flasks at 39 ± 1 °C. The ruminal fluids were filtered under CO_2 gas pressure through four layers of muslin cloth and were used for the *in vitro* gas production. *Origanum syriacum* L. volatile oil was drawn into 100 ml glass syringes (FORTUNA®; Merck KGaA, Darmstadt, Germany) and added at levels of 40/60/80 mg/l to the ruminal fluid samples. Carvacrol (Sigma Aldrich Co., St. Louis, MO, USA) was added to the ruminal liquid samples at a level of 60 mg/l. Incubation was performed in 10 ml sample aliquots with 200 ± 10 mg of dried lucerne herbage and 20 ml of a mixture of buffer + macrominerals + microminerals + reduction solution + resazurin solution. Gas was produced in four replicates of the samples from each group. Four syringes were used for the blind calculations.

Determination of the *in vitro* total gas and methane production

The total amount of gas produced in each syringe was determined by reading the volume (ml) on the syringe barrel at the end of a 24 h-incubation period. The share of methane in the total percentage (%) of gas produced was determined using an infrared methane sensor (Sensor Europe GmbH, Erkrath, Germany).

Determination of the *in vitro* degradability parameters

The effects of *Origanum syriacum* L. and carvacrol on the *in vitro* metabolisable energy (ME), organic matter digestibility (OMD) and net energy lactation (NE_L) values were calculated using the formulae indicated below (Menke et al. 1979; Blummel et al. 1997).

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \times GP + 0.057 \times CP + 0.002\,859\,7 \times EE^2 \quad (1)$$

$$\text{OMD (\% DM)} = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.065\,1 \times CA \quad (2)$$

where:
DM – dry matter;
GP – 24 h net gas production (ml/200 mg, DM);
CP – crude protein (g/kg DM);
CA – crude ash (g/kg DM);
EE – ether extract (g/kg DM).

Determination of the total protozoa count

At the end of the incubation period, the content of the glass syringes was used to count the number of protozoa. At the end of 24 h, 1 ml of the content

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was filtered from each syringe and 49 ml of a diluent was added (mixture of 20 ml of 37% formalin, 150 ml of glycerine and 820 ml of distilled water) to prepare the ready-to-count 50 ml sample aliquots as described by Boyne et al. (1957). One ml of each aliquot was placed in the chamber of a McMaster's slide to count the number of protozoa per cubic centimetre (Boyne et al. 1957).

Statistical analysis

The statistical analyses of the raw data obtained in this study were made using the SPSS v17.0 (IBM, USA) software package. The statistical significance of the groups was determined by a one-way analysis of variance (ANOVA). The dose-dependent differences of the *in vitro* digestion parameters were detected with a polynomial contrast analysis (linear, quadratic and cubic). When a statistical significance was detected, Tukey's multiple range test was performed as a multiple comparison test. The *P*-level for statistical significance was set below 0.05 ($P < 0.05$).

Table 1. Chemical components of the *Origanum syriacum* L. volatile oil (%)

Components	Rate (%)	Retention time (RT)
α-Pinene	1.30	12.81
α-Thujene	1.62	13.09
β-Pinene	0.27	17.38
Myrcene	2.95	21.14
α-Terpineol	4.47	22.08
D-Limonene	0.59	23.18
Sabinene	0.41	23.77
γ -Terpinene	21.89	26.70
p-Cymene	17.90	28.38
Octan-3-ol	2.75	36.16
trans-Sabinene hydrate	2.04	40.73
Caryophyllene	2.21	48.79
3-Cyclohexen-1-ol	1.27	49.21
Humulene	0.31	52.89
Terpineol	0.31	54.46
Borneol	0.37	54.74
β-Caryophyllene oxide	0.50	65.67
Thymol	19.38	69.48
Carvacrol	19.23	70.39

RESULTS

The chemical composition of the volatile oil extracted by steam distillation is shown in Table 1. The main constituents of the *Origanum syriacum* L. volatile oil, determined by chemical analysis, include γ-terpinene (21.89%), thymol (19.38%), carvacrol (19.23%) and *p*-cymene (17.90%).

The effects observed at the end of a 24 h-incubation period on the *in vitro* gas production, ammonia nitrogen level and protozoa counts are listed in Table 2. The results obtained in the present study demonstrated that carvacrol and the different doses of OSVO have significantly altered gas production. It was determined that, when compared to the control group, the gas produc-

Table 2. The effect of *Origanum syriacum L.* and carvacrol on the in vitro digestion parameters of lucerne herbage

Parameter	Control	CRV (60 mg/l)	OSVO (40 mg/l)	OSVO (60 mg/l)	OSVO (80 mg/l)	SEM	*P*-value
GP (ml/g)	39.30 ± 1.80^{a}	33.60 ± 0.62^{b}	36.16 ± 0.44^{a}	35.72 ± 1.30^{b}	36.53 ± 1.24^{a}	0.871	*
CH_4 (%)	21.95 ± 0.20^{b}	23.55 ± 0.52^{ab}	22.67 ± 0.51^{ab}	24.02 ± 0.97^{ab}	25.07 ± 0.37^{a}	0.337	*
Methane (ml)	8.63 ± 0.45^{ab}	6.96 ± 0.19^{b}	8.19 ± 0.15^{ab}	8.60 ± 0.60^{ab}	9.16 ± 0.41^{a}	0.233	*
ME (MJ/kg DM)	8.58 ± 0.24^{a}	7.26 ± 0.08^{b}	8.16 ± 0.06^{a}	8.10 ± 0.16^{a}	8.21 ± 0.16^{a}	0.118	*
OMD (%)	64.43 ± 1.760^{a}	55.81 ± 0.55^{b}	61.64 ± 0.38^{a}	61.25 ± 1.16^{a}	61.97 ± 1.10^{a}	0.774	*
NH_3-N (mg/l)	477.5 ± 2.5^{a}	410 ± 11.9^{c}	417.5 ± 4.7^{bc}	425.0 ± 9.1^{bc}	455.0 ± 15.8^{ab}	7.05	*
Protozoa ($\times 10^3$/ml)	2.70 ± 0.10	3.08 ± 0.12	2.82 ± 0.11	3.07 ± 0.16	2.67 ± 0.19	0.069	NS

*Differences between the averages values indicated by different letters in the same row are important ($P < 0.01$)

CH_4 = methane production as a percentage of the total gas production; CRV = carvacrol; GP = 24 h gas production; ME = metabolic energy; NH_3-N = nitrogen bound to ammonia; NS = not significant; OMD = organic matter digestibility; OSVO = *Origanum syriacum* L. volatile oil

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Table 3. The effect of *Origanum syriacum* L. and carvacrol on athe molarities of volatile fatty acids in the *in vitro* fermentation fluid (mmol/l)

Volatile fatty acids	Control	CRV (60 mg/l)	OSVO (40 mg/l)	OSVO (60 mg/l)	OSVO (80 mg/l)	SEM	*P*-value
TVFA	102.96 ± 0.86^{a}	98.28 ± 0.77^{d}	99.92 ± 0.83^{b}	99.35 ± 0.96^{b}	98.94 ± 0.81^{c}	1.204	**
AA	56.07 ± 1.30	53.04 ± 0.81	54.03 ± 1.16	53.03 ± 1.29	53.04 ± 1.24	1.656	NS
PA	21.04 ± 1.64	20.05 ± 2.54	20.04 ± 1.61	19.04 ± 1.54	20.06 ± 2.70	1.464	NS
BA	18.05 ± 0.97^{ab}	17.03 ± 1.16^{b}	18.05 ± 1.19^{ab}	20.34 ± 0.95^{a}	20.04 ± 1.06^{a}	1.512	*
OFA	4.83	4.88	4.86	3.89	2.87	1.741	NS
AA/PA	2.66	2.67	2.86	2.95	2.78	0.194	NS

*Differences between the average values indicated by different letters in the same row are important; **Differences between the average values indicated by different letters in the same row are statistically significant ($P < 0.05$)

AA = acetic acid; AA/PA = acetate/propionate ratio; BA = butyric acid; CRV = carvacrol; OFA = other fatty acids comprised of iso-butyrate + valerate + iso-valerate; OSVO = *Origanum syriacum* L. volatile oil; PA = propionic acid; TVFA = (as mmol/l rumen fluid) total volatile fatty acids comprised of acetate + propionate + butyrate + iso-butyrate + valerate + iso-valerate

tion decreased by 9% with the addition of 60 mg/l of OSVO, and by 14% with the addition of carvacrol ($P < 0.01$). The methane production (ml), organic matter degradability (OMD) and metabolisable energy (ME) were observed to have decreased with the addition of carvacrol ($P < 0.01$). Compared to the control group, the ruminal ammonia levels were observed to have significantly decreased in all of the treatment groups ($P < 0.01$).

As seen in Table 3 which presents the effects of the carvacrol and *Origanum syriacum* L. volatile oil on the ruminal fermentation, while the TVFA concentration significantly decreased with the addition of carvacrol and all the doses of *Origanum syriacum* L. volatile oil ($P < 0.05$), the BA level decreased with the addition of carvacrol only ($P < 0.01$). The addition of 80 mg/l of *Origanum syriacum* L. volatile oil and 60 mg/l of carvacrol were found to be the most effective on the ruminal fermentation.

DISCUSSION

The effects and compositional levels of the secondary plant components vary with the species, geographical region and location. The effect of a plant extract is related to the antimicrobial, antiprotozoal and antioxidant substances the plant contains (Onel et al. 2020). Gas production during *in vitro* incubation is generally a good sign of ruminal degradation and microbial activity, and higher gas production levels indicate the presence of better nutritional sources for ruminal microorganisms (Boussaada et al. 2018). To date, the antimicrobial, antiprotozoal and antioxidant effects of plant volatile oils have been extensively investigated, yet the effects of these oils on the methane emissions have not been determined. The volatile oil used in this study, which was extracted from *Origanum syriacum* L., a plant that grows along the Mediterranean coastline, is known to show antibacterial effect owing to the high levels of γ-terpinene (21.89%), thymol (19.38%), carvacrol (19.23%) and *p*-cymene (17.90%) it contains (Onel and Aksu 2020). The volatile oil levels and chemical composition previously reported for plants harvested from similar locations are largely in agreement with those determined for the OSVO in the present study (Karik et al. 2015; Aksu et al. 2018). On the other hand, in a study on a single plant species from three different locations, Cook et al. (2007) determined differences in the composition of the volatile oils extracted from the specimens of the plant. These differences were not directly related to the prevailing climate conditions, but were rather of a local character and were observed for the different harvesting regions and plant organs.

Plant extracts intended to be used as feed additives and their administration doses should be selected in such a way that they do not show any adverse effect on the ruminal fermentation and do not cause feed spoilage. Plant volatile oils should improve the reduction in the ruminal ammonia concentrations (Patra and Yu 2012; Karik et al. 2015; Onel et al. 2020). In the present study, 60 mg/l of *Origanum*

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syriacum L. volatile oil and 60 mg/l of carvacrol added to the ruminal fluid samples were determined to have reduced the *in vitro* gas production ($P < 0.01$). The differences observed between the effects of the other *Origanum syriacum* L. volatile oil doses on CH_4 production were attributed to the differences in the levels of the phenolic compounds these doses contained. On the other hand, previous studies reporting increased gas production during the *in vitro* incubation with extracts of plants rich in secondary metabolites, including *Origanum syriacum* L., have attributed this increase to the secondary metabolites, and, in particular, to flavonoids (Jimenez-Peralta et al. 2011; Sallam et al. 2011).

In agreement with the reports of Mandal et al. (2016) and Onel et al. (2020), the present study demonstrated that carvacrol and *Origanum syriacum* L. volatile oil both showed a positive effect by reducing the ruminal ammonia levels without altering the protozoal counts. In view of the degradability, protozoal growth and reduced ammonia levels reported to being inhibited by the secondary plant metabolites (Patra and Yu 2014), the nitrogen decreasing effect of *Origanum syriacum* L. was attributed to the inhibition of the growth of ammonia-producing bacteria. On the other hand, different from *in vitro* research, other studies are available, which have been carried out in dairy cattle and sheep and suggest that volatile oils and the active substances they contain, such as carvacrol, do not affect the enteric methane production and bacterial nitrogen concentration. The TVFA and PA concentrations in the *in vitro* fermentation fluid of lucerne herbage were low in all the groups, while a low level of butyric acid was detected only in the group where carvacrol was added. It was ascertained that *Origanum syriacum* L. volatile oil led to dose-dependent alterations in the *in vitro* degradation parameters. In result, the addition of 40 mg/l of *Origanum syriacum* L. volatile oil and 60 mg/l of carvacrol to the ruminal fluid led to a decreasing effect in the *in vitro* ruminal gas and methane production.

The anti-methanogenic effect of the *Origanum syriacum* L. volatile oil was due to the decreased digestibility of the lucerne herbage. This can have a positive impact on the environment, but the same cannot be said for the digestibility of all the nutrients and the animal performance. For this reason, there is a need for *in vivo* studies that more clearly reveal the mode of action of plant extracts on reducing the methane production, and on determining the relationships between the composition of the diet and the dose of the plant extract used. Thus, although it is not certain, it will be possible to test the view that some plant extracts can reduce the palatability of the feeds and the feed intake.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

AOAC – Association of Official Analytical Chemists. Official methods of analysis of AOAC international. Washington, DC: Association of Official Analytical Chemists; 1995.

Aksu T, Saripinar AD, Kaya DA, Duran N, Onel SE, Canogullari S. The response of Japanese quails to dietary Thymbra spicata L. essential oil. Rom Biotech Lett. 2018;23(5):13909-17.

Baytok E, Kara K, Aksu T, Guclu BK, Ozkaya S, Denek N, Akcay A. The effect of Mediterranean thyme (Thymbra spicata L. var. spicata) essential oil on fattening performance and ruminal parameters in lamb. J Anim Feed Sci. 2017;26(4):319-25.

Blummel M, Makkar HPS, Becker K. In vitro gas production: A technique revisited. J Anim Physiol Anim Nutr. 1997;77(1):24-34.

Boussaada A, Arhab R, Calabro S, Grazioli R, Ferrara M, Musco N, Cutrignelli MI. Effect of Eucalyptus globulus leaves extracts on in vitro rumen fermentation, methanogenesis, degradability and protozoa population. Ann Anim Sci. 2018;18(3):753-67.

Boyne AW, Eadie JM, Raitt K. The development and testing of a method of counting rumen ciliate protozoa. J Gen Microbiol. 1957 Oct;17(2):414-23.

Castaneda-Correa A, Corral-Luna A, Hume ME, Anderson RC, Ruiz-Barrera O, Castillo-Castillo Y, Rodriguez-Almeida F, Salinas-Chavira J, Arzola-Alvarez C. Effects of thymol and carvacrol, alone or in combination, on fermentation and microbial diversity during in vitro culture of bovine rumen microbes. J Environ Sci Health B. 2019;54(3):170-5.

Cobellis G, Trabalza-Marinucci M, Marcotullio MC, Yu Z. Evaluation of different essential oils in modulating methane and ammonia production, rumen fermentation, and rumen bacteria in vitro. Anim Feed Sci Technol. 2016; 215:25-36.

https://doi.org/10.17221/35/2021-VETMED

Cook CM, Maloupa E, Kokkini S, Lanaras T. Differences between the inflorescence, leaf and stem essential oils of wild Mentha pulegium plants from Zakynthos, Greece. J Essent Oil Res. 2007;19(3):239-43.

Gerber PJ, Hristov AN, Henderson B, Makkar H, Oh J, Lee C, Meinen R, Montes F, Ott T, Firkins J, Rotz A, Dell C, Adesogan AT, Yang WZ, Tricarico JM, Kebreab E, Waghorn G, Dijkstra J, Oosting S. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: A review. Animal. 2013 Jun;7(Suppl_2): 220-34.

Hristov AN, Oh J, Firkins JL, Dijkstra J, Kebreab E, Waghorn G, Makkar HP, Adesogan AT, Yang W, Lee C, Gerber PJ, Henderson B, Tricarico JM. Special topics – Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J Anim Sci. 2013 Nov;91(11):5045-69.

IPCC. Climate change: Impacts, adaptation, and vulnerability. Part B: Regional aspects. Contribution of working group II to the fifth assessment report of the inter-governmental panel on climate change. New York, NY, USA: Cambridge University Press; 2014. 688 p.

Jafari S, Ebrahimi M, Goh YM, Rajion MA, Jahromi MF, Al-Jumaili WS. Manipulation of rumen fermentation and methane gas production by plant secondary metabolites (saponin, tannin and essential oil) – A review of ten-year studies. Ann Anim Sci. 2019;19(1):3-29.

Jimenez-Peralta FS, Salem AZM, Mejia-Hernandez P, Gonzalez-Ronquillo M, Albarran-Portillo B, Rojo-Rubio R, Tinoco-Jaramillo JL. Influence of individual and mixed extracts of two tree species on in vitro gas production kinetics of a high concentrate diet fed to growing lambs. Livest Sci. 2011;136(2-3):192-200.

Karik U, Cicek F, Tutar M, Ayas F. Essential oil compounds of Turkey laurel (Laurus nobilis L.) populations. Anadolu J AARI. 2015;25(1):1-16.

Mandal GP, Roy A, Patra AK. Effects of plant extracts rich in tannins, saponins and essential oils on rumen fermentation and conjugated linoleic acid conentrations in vitro. Indian J Anim Hlth. 2016;55(1):49-60.

Menke KH, Raab L, Salewsk A, Steingass H, Fritz D, Schneide W. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. J Agric Sci Comb. 1979;93(1):217-22.

Onel SE, Aksu T. Can thyme (Thymbra spicata L. var. spicata) volatile oil alleviate the detrimental effects of high stocking densities in Japanese quail? Turk J Vet Anim Sci. 2020;44(5):1024-31.

Onel SE, Aksu T, Kara K, Aksu DS. Effects of essential oils derived from different thyme species on in vitro nutrient digestibility, rumen protozoa number and methane production of alfalfa hay. 2nd International Animal Nutrition Congress Antalya, Turkey, November 1-4; 2018. p. 108-12.

Onel SE, Aksu T, Kara K, Aksu DS. The effects of Laurel volatile oil (Laurusnobilis L.) on in vitro ruminal gas production of methane emission, organic acids and protozoa counts alfalfa herbage. Erciyes Üniv Vet Fak Derg. 2020; 17(3):283-9.

Patra AK, Yu Z. Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Appl Environ Microbiol. 2012 Jun;78(12):4271-80.

Patra AK, Yu Z. Effects of vanillin, quillaja saponin, and essential oils on in vitro fermentation and protein-degrading microorganisms of the rumen. Appl Microbiol Biotechnol. 2014 Jan;98(2):897-905.

Sallam SM, Abdelgaleil SA, Bueno IC, Nasser ME, Araujo RC, Abdalla AL. Effect of some essential oils on in vitro methane emission. Arch Anim Nutr. 2011 Jun;65(3): 203-14.

Soltan Y, Morsy AS, Araujo RC, Elziat HM, Sallam SMA, Louvandini H, Abdalla AL. Carvacrol and eugenol as modifiers of rumen microbial fermentation, and methane production in vitro. Proceedings of 4th Animal Wealth Research Conference in the middle East and North Africa; Egypt, Oct 3-5; 2011. p. 354-64.

Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991 Oct;74(10):3583-97.

Wanapat M, Cherdthong A, Phesatcha K, Kang S. Dietary sources and their effects on animal production and environmental sustainability. Anim Nutr. 2015 Sep;1(3): 96-103.

Zhou R, Wu J, Lang X, Liu L, Casper DP, Wang C, Zhang L, Wei S. Effects of oregano essential oil on in vitro ruminal fermentation, methane production, and ruminal microbial community. J Dairy Sci. 2020 Mar;103(3):2303-14.

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