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Süt İneği Toplam Rasyon Karışımının *İn Vitro* Ruminal Gaz Üretimi, Metan Salınımı, Rumen Uçucu Yağ Asitleri Miktarı ve Protozoa Sayısı Üzerine *Aquilaria agallocha Roxb.* Uçucu Yağının Etkisi

Süleyman Ercüment ÖNEL[1*], Kanber KARA[2], Mehmet GÜL[3], Mehmet Akif ÖZTAŞ[4], Sena YILMAZ[5]

[1]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Hatay-Turkey
[2,4,5]Erciyes University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Kayseri-Turkey
[3]Ataturk University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Erzurum-Turkey

[1]https://orcid.org/0000-0001-6599-0541
[2]https://orcid.org/0000-0001-9867-1344
[3]https://orcid.org/0000-0001-5477-1773
[4]https://orcid.org/0000-0002-9937-0719
[5]https://orcid.org/0000-0002-0161-4923
*Sorumlu yazar: ercumentonel@gmail.com

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ÖZET

Ruminal fermantasyon sonucu oluşan ve sera gazı olan metan (CH_4) hem ekonomik hem de ekolojik olarak küresel iklim değişikliğinde önemli paya sahiptir. Ruminant beslemede uçucu yağların metan emisyonlarını azalttığına yönelik çalışmalar bulunmaktadır. Bu *in vitro* çalışmanın amacı, Aquilaria agallocha Roxb. bitkisinden elde edilen uçucu yağın yonca kuru otunun ruminal gaz üretimi, metan emisyonu, rumen organik asitleri miktarı ve protozoa sayısı üzerine etkilerini araştırmaktır. Çalışmada, Aquilaria agallocha Roxb. uçucu yağının bileşenleri analiz edilmiştir. Rumen sıvısına 5, 10 ve 15 µL/0,2 g kuru madde (KM) (A0, A5, A10 ve A15) seviyelerinde Aquilaria agallocha Roxb. uçucu yağı eklenerek *in vitro* ruminal sindirime etkileri *in vitro* gaz üretimi ile belirlenmiştir. Sonuç olarak rumen sıvısına 10 µL Aquilaria agallocha Roxb. uçucu yağı eklenmesinin, süt ineği toplam rasyon karışımının (TMR) *vitro* ruminal gaz üretimi ve sindirim parametreleri üzerine olumlu etki göstererek antimetanojenik etki gösterdiği belirlenmiştir.

The Effects of *Aquilaria agallocha Roxb.* Volatile Oil on *In Vitro* Ruminal Gas Production, Methane Emission, Volatile Fatty Acide Amounts and Protozoa Counts of Total Mixed Ration of Dairy Cattle

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ABSTRACT

Methane (CH_4), a major greenhouse gas produced during ruminal fermentation, has a significant ratio in both the economic and ecological impact of global climate change. Literature reports have been published, which suggest that dietary supplementation with plant volatile oils reduces methane emissions in ruminant production. This *in vitro* study was aimed at investigating the effects of agarwood (Aquilaria agallocha Roxb.) volatile oil on *in vitro* ruminal gas production, methane emission, ruminal organic acids and protozoa counts of total mixed ration (TMR) of dairy cattle. Component analyses were performed for agarwood (Aquilaria agallocha Roxb.) volatile oil. The effects of agarwood volatile oil, added to ruminal fluid at levels of 5, 10 and 15 µL/0.2 g dry matter (DM) (Groups A0, A5, A10 and A15), on *in vitro* ruminal digestion were determined based on *in vitro* gas production. In conclusion, it was ascertained that the addition of 10 µL of Aquilaria agallocha Roxb. volatile oil to ruminal fluid positively affected *in vitro* ruminal gas production and digestion parameters of total mixed ration (TMR) of dairy cattle, and showed an antimethanogenic effect.

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Introduction

In view of the ever-increasing global population, increased income-purchasing power, and urbanization trend, it is predicted that, until 2050, the global demand for meat and milk will increase by 73% and 58%, respectively, compared to the 2010 levels (Beauchemin, 2020). Owing to its contribution to both the increasing atmospheric greenhouse gas concentrations and climate change, the increasing level of animal production is of concern. Global greenhouse gas emissions caused by ruminant production (animals, manure, feed production and deforestation for land clearing) are estimated to account for 14.5% of total anthropogenic emissions (Gerber et al., 2013, Schultz et al., 2020). In this context, producers are in need of cost-effective methods that would reduce greenhouse gas emissions while meeting the demand for high quality, safe and affordable food produced from healthy animals.

The meat and milk of ruminants are major protein sources for human consumption. Ruminants offer a unique advantage of being able to feed on nonarable meadows and pastures yet, 2-12% of the gross amount of energy they consume is converted to CH_4 during ruminal digestion (Onel et al., 2020). Methane (CH_4), which is a major greenhouse gas produced during ruminal fermentation, has a significant ratio in the adverse economic and ecological impact of global climate change. Reports indicate that plant volatile oils show an antimethanogenic effect on *in vitro* digestion and alter ruminal fluid parameters (Cobellis et al., 2016; Ulger et al., 2017; Zhou et al., 2020).

This study was designed in view of the possibility of agarwood (Aquilaria agallocha Roxb.) volatile oil also altering ruminal fluid parameters and *in vitro* digestion owing to the active substances it contains.

Materials and Methods

Composition of total mixed ration

The agarwood (Aquilaria agallocha Roxb.) volatile oil used in this study was supplied from a commercial company. The dairy cattle ration was composed of 25.39% corn silage, 13.12% wheat straw, 19.96% dried alfalfa, 15.73% barley, 8.29% sunflower meal 8.71% cottonseed meal and 8.29% wheat bran. These rates indicate the dry matter percentages of the feedstuffs. The crude protein (CP), net energy lactation (NEL), neutral detergent fiber (NDF) and non-fiber carbohydrate (NFC) content of the dairy cattle ration were 142 g kg^{-1} DM,1.34 Mcalkg^{-1} DM, 456 g kg^{-1} DM and 324 g kg^{-1} DM, respectively.

Chemical analyses of the Aquilaria agallocha Roxb. volatile oil

The agarwood (Aquilaria agallocha Roxb.) volatile oil used in this study was supplied from a commercial company. The chemical components of the volatile oil were determined using a Thermo Scientific ISQ Single Quadrupole model gas chromatograph and a TG-Wax MS-A model, 5% phenyl polysilphenylene-siloxane column of 0.25 mm inner diameter, 30 m length, and 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 to 1200 amu. The scan mode was used for 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 and mass spectra.

In vitro gas production

The *in vitro* digestibility of TMR was determined by the *in vitro* gas production assay described by Menke et al. (1979). Approximately 1-litre ruminal fluid samples were collected from each of two fistulated 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 4 layers of muslin cloth and were used for *in vitro* gas production. Aquilaria agallocha Roxb. volatile oil was drawn into 100 ml-glass syringes (Model Fortuna, Germany), added at levels of 5-10-15 µL to the ruminal fluid samples, and then incubated in 10 ml-aliquots with 200±10 mg of TMR 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 blind calculations.

Determination of 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 ratio of methane in the total amount of gas produced was determined using an infrared methane sensor (Sensor, Europe GmbH, Erkrath, Germany).

Determination of in vitro digestibility parameters

The effects of Aquilaria agallocha Roxb. volatile oil on the *in vitro* metabolizable energy (ME), organic matter digestibility (OMD) and NEL values of TMR were calculated using the formulae indicated below (Blümmel et al., 1997; Menke et al., 1979).

ME (MJ /kg DM) = 2.20 + 0.136 × GP + 0.057 × CP + 0.0028597 x EE2

OMD (g/kg DM) = 14.88 + 0.889 × GP+ 0.45 × CP + 0.0651 × CA

GP = 24 h net gas production (ml/200 mg), CP = Crude protein (g/kg DM), CA = Crude ash (g/kg DM), EE = Ether extract (g/kg DM).

Determination of total protozoa number

At the end of the incubation period, the content of *in vitro* fermentation fluid in glass syringes was used to determine the number of total protozoa (Dehority, 1984).

Determination of pH and ammonia concentration

The pH value of the filtered *in vitro* fermentation fluid was determined using a digital pH meter (Mettler Toledo S220, Switzerland). The ammonia concentration (mg/dl) in the rumen fluid was determined using a commercial ammonia assay procedure (Megazyme, K-AMIAR 02/20, Wicklow, Ireland) (Kara, 2021).

Determination of organic acids in the in vitro digestion fluid

To determine *in vitro* rumen organic acids amounts, after 24 h of *in vitro* incubation, the total gas volume was recorded. Ten ml of the digestion fluid was transferred into Falcon tubes, a gas chromatography (GC) device (Thermo Trace 1300, Thermo Scientific, Waltham, MA, USA). This device was equipped with a polyethylene glycol column (inner diameter: 0.25 mm, length: 60 m, film thickness: 0.25 µm) (TG-WAXMS, Thermo Scientific, Waltham, MA, USA) and a flame ionisation detector (FID). The GC device was operated in accordance with the procedure described by Ersahince and Kara (2017).

Statistical analyses

Statistical analyses were performed by SPSS 20.0 (IBM Inc, Chicago, IL, USA). The descriptive statistics were presented as mean±SEM for measurements. The study groups were compared by one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) post-hoc test. Significant pairwise differences were indicated in the tables. P value of less than 0.05 was considered as a statistically significant.

Results

The chemical composition of the agarwood (Aquilaria agallocha Roxb.) volatile oil used in this study is presented in Table 1. The major components of the volatile oil, in order of ratio were determined as follows: methyl stearate (32.07%), 9,.12-octadecadienoic acid (19.21%), vaccenic acid (12.16%), palmitic acid (9.98%), and 9,.12-octadecadienoic acid (7.46%).

The effects of the different doses of Aquilaria agallocha Roxb. volatile oil on *in vitro* gas production, ammonia nitrogen levels, and protozoa counts by the end of a 24 h-incubation period are presented in Table 2. Study group comparisons showed that the amount of total gas production was 13.20±6.64 ml/0.2 g in Group A10 and significantly differed from that in the control group (37.07±3.28 ml/0.2 g) (p=0.002). No statistically significant difference was determined between the groups for methane percentages. Methane production was lowest in Group A10 and highest in the control group (p=0.003).

Table 1. Chemical components of agarwood (Aquilaria agallocha Roxb.) volatile oil

Retention Time (RT) (min)	Rate (%)	Components
11.82	0.28	(z)-2-decenal
12.91	0.12	(e,z)-2,4-decadienal
16.06	0.11	1-ethyl-1-methylcyclohexane
18.92	0.05	(-)-alpha-curcumene
19.09	0.09	cis-sesquisabinene hydrate
24.47	0.11	cedr-8-en-13-ol
27.44	0.13	andrographolide
29.20	0.62	callitrin
30.95	1.21	eudesma-5,11(13)-dien-8,12-olide
31.28	6.07	methyl palmitate
32.06	1.78	androstan-17-one,3-ethyl-3-hydroxy (5alpha)
32.73	9.98	palmitic acid
33.74	0.27	isopropyl palmitate
35.46	7.46	9,12-octadecadienoic acid
35.61	19.21	9-octadecenoic acid (Z)-, methyl ester
35.74	2.39	methyl octadec-10-enoate
36.20	3.27	methyl stearate
37.23	32.07	vaccenic acid
37.57	12.16	stearic acid
38.77	1.3	9,12-octadecadienoic acid
40.70	0.48	methyl arachidate
41.32	0.32	delta-octadecalactone
41.68	0.52	9-octadecenamide

While ME was low in Group A10, it was measured at a significantly higher level in the control group (p=0.002). OMD measurements displayed a significant decrease between Groups A5 and A10, and significant increases between Group A10 and the control group, as well as between Group A15 and the control group (p=0.002). NH_3-N measurements significantly differed between Groups A10, A5 and A15. Furthermore, a significant increase was detected between Group A10 and the control group (p=0.002). While protozoa counts were observed to have increased in Group A15 and the control group, the differences between the groups were statistically insignificant (Table 2).

The types of organic acids in the digestion fluid were compared between the treatment groups and control group (Table 3). It was ascertained that the study groups significantly differed for all organic acid types, excluding valeric acid, as well as for the acetate: proprionate ratio (AA/PA) and total VFA concentration ($p<0.01$). For all statistically significant differences between the study groups, the control group displayed significantly higher values than Groups A5, A10 and A15. Close values measured in the treatment groups did not display any statistically significant difference.

Discussion

The levels and activity of secondary phytocomponents vary with plant species and the harvest area and location. The efficacy of plant extracts arises from the antimicrobial, antiprotozoal and antioxidant substances they contain.

Table 2. The effects of Aquilaria agallocha Roxb. volatile oil on the *in vitro* digestion parameters of TMR

Groups	TGP (ml/0.2 g DM)	Methane (%)	Methane (ml)	ME (MJ/kg DM)	OMD (%)	pH	NH3-N (mg/dl)	Protozoa ($x10^4$/ml)
A0 (control)	37.07±1.89	23.63±0.27	8.76±0.42	7.93±0.25	53.76±1.68	5.55±0.03	75.53±0.70	8.11±0.94
A5	29.37±2.54	24.83±0.49	7.31±0.76	6.89±0.35	46.92±2.29	5.63±0.09	74.28±1.07	2.32±0.35
A10	13.20±3.83	25.47±1.39	3.37±1.00	4.69 ±0.52	32.54 ±3.40	5.57±0.01	54.34±1.33	2.21±0.23
A15	16.86±3.43	25.43±1,66	4.22±0.70	5.19±0.46	35.80±3.05	5.73±0.04	74.77±1.07	6.44±1.42
p-values	0.002*	0.644	0.003*	0.002*	0.002*	0.164	0.002*	0.171
Post-hoc	A0>A10 A0>A15 A5>A10		A0>A10 A0>A15 A5>A10	A0>A10 A0>A15 A5>A10	A0>A10 A0>A15 A5>A10		A0>A10 A5>A10 A15>A10	

A0, A5, A10, A15: Groups added 0 (control), 5, 10, and 15 µL of *Aquilaria agallocha* Roxb. volatile oil, respectively.
TGP: Total gas production (24h ml/ 0.2 g DM), ME: Metabolic energy as MJ/kg DM, OMD: Organic matter digestibility as %. *: p<0.05

Gas production during *in vitro* incubation is generally a good indicator of ruminal digestion and ruminal microbial activity, such that higher gas production levels indicate better nutritional sources for the rumen microbiota (Makkar et al., 1997, Tural and Turhal 2017). While plant volatile oils have been extensively investigated for their antimicrobial, antiprotozoal and antioxidant effects, their potential effects on methane emission remain unknown.

Plant extracts and their administration doses should be selected with a view to avoid any adverse effect on ruminal fermentation and feed deterioration. The use of plant volatile oils should result in a positive effect on the reduction of ruminal ammonia concentrations. While literature information available on the effects of agarwood (*Aquilaria agallocha* Roxb.) volatile oil on rumen parameters is scarce, the agarwood volatile oil used in the present study was determined to show a positive effect by reducing ruminal ammonia concentrations without altering ruminal protozoa counts. The 10 µL-dose of *Aquilaria agallocha* Roxb. volatile oil was ascertained to have reduced gas production ($p < 0.01$). Accordingly, the differences between the other administration doses of agarwood volatile oil for methane production could be attributed to the concentrations of antibacterial compounds found in these doses. On the other hand, literature reports indicating an increased gas production during *in vitro* incubation for various secondary metabolite-rich plant extracts, including that of *Aquilaria agallocha* Roxb., have linked this increase to the adverse effects of the secondary metabolites found in these extracts (Joch et al., 2016, Garcia et al., 2020).

Table 3. The effects of Aquilaria agallocha Roxb. volatile oil on ruminal fermentation parameters

	A0 (control)	A5	A10	A15	P-values	Post-hoc
TVFA	97.61±0.55	68.35±1.21	61.40±0.94	66.97±1.47	<0.001*	A0>A5 A0>A10 A0>A15
AA	66.61±0.95	45.93±2.11	41.5±1.63	45.35±2.56	<0.001*	A0>A5 A0>A10 A0>A15
PA	13.09±0.11	10.18±0.21	10.05±0.23	10.3±0.23	<0.001*	A0>A5 A0>A10 A0>A15
BA	13.49±0.29	8.88±0.30	7.32±0.32	8.33±0.49	<0.001*	A0>A5 A0>A10 A0>A15
IBA	1.03±0.01	0.74±0.05	0.55±0.07	0.65±0.06	0.002*	A0>A5 A0>A10 A0>A15
IVA	2.08±0.03	1.40±0.14	0.97±0.18	1.20±0.13	0.003*	A0>A5 A0>A10 A0>A15
HA	0,.6±0.006	0.23±0.006	0.20±0.014	0.22±0.014	<0.001*	A0>A5 A0>A10 A0>A15
VA	0.95±0.31	1.00±0.05	0.81±0.07	0.92±0.06	0.880	
AA/PA	5.08±0.01	4.51±0.16	4.13±0.3	4.40±0.09	0.001*	A0>A5 A0>A10 A0>A15

A5, A10, A15: Groups added 5, 10 and 15 µL of Aquilaria agallocha Roxb. volatile oil, respectively, to rumen fluid. TVFA: (as mmol/L rumen fluid) total volatile fatty acids comprise of acetate + propionate + butyrate + iso-butyrate + valerate + iso-valerate; AA: acetic acid, PA: propionic acid, BA: butyric acid, IBA: Isobutyric acid, IVA: Isovaleric acid, HA: Hexanoic acid, VA: Valeric acid, AA/PA: acetate/propionate.
*: p<0.05

The positive effect achieved in the present study with the use of *Aquilaria agallocha* Roxb. volatile oil, observed as reduced ruminal ammonia, methane (ml) and total gas levels with no alteration in the protozoal population and pH level, agrees with the reports of Mandal (2016) and Onel (2020). In view of reports indicating the inhibition of digestibility, protozoal growth, and reduced ammonia concentrations by plant secondary compounds (Patra and Yu, 2012), the reducing effect of *Aquilaria agallocha* Roxb. volatile oil on nitrogen and methane (ml) levels is considered to be related to the inhibition of ammonia-producing bacteria.

In all groups, the concentrations of volatile fatty acids, excluding valeric acid, in the *in vitro* fermentation fluid of TMR, were low. The concentrations of acetic, proprionic and butyric acids were low in Group A10. We determined that agarwood (*Aquilaria agallocha* Roxb.) volatile oil led to dose-dependent alterations in the *in vitro* digestion parameters.

In conclusion, the addition of 10 µL of *Aquilaria agallocha* Roxb. volatile oil to the ruminal fluid was observed to have positively affected the *in vitro* ruminal gas production and digestion parameters of total mixed ration of dairy cattle, and to have shown an antimethanogenic effect. Thus, there is need for further investigation on the adaptation of the positive effects of plants and plant extracts to *in vivo* conditions in long-term studies.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Ethical approval

This study was conducted pursuant to the approval (No: 2021/04-16) of the Local Ethics Committee of Hatay Mustafa Kemal University.

Author's Contributions

The contribution of the authors is equal.

References

Beauchemin KA., Ungerfeld EM., Eckard RJ., Wang M. Review: fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation. Animal 2020; 14(1): 2-16.

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

Dehority BA. Evaluation of subsampling and fixation procedures used for counting rumen protozoa. Appl Environ Microbiol 1984; 48: 182-185.

Cobellis G., Trabalza MM., 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 Technology 2016; 215: 25-36.

Ersahince C., Kara K. Nutrient composition and *in vitro* digestion parameters of Jerusalem artichoke (*Helianthus tuberosus* L.) herbage at different maturity stages in horse and ruminant. Journal of Animal and Feed Sciences 2017; 26(3): 213-225.

Garcia F., Colombatto D., Brunetti MA., Martínez MJ., Moren MV., Scorcione TM., Martínez FJ. The reduction of methane production in the *in vitro* ruminal fermentation of different substrates is linked with the chemical composition of the essential oil. Animals 2020; 10(5): 786.

Gerber P., Hristov AN., Henderson B., Makkar H., Oh J., Lee C., Meinen R., Montes F., Ott T., Firkins J., A Rotz., 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; 2(2): 220-234.

Ulger I., Kamalak A., Kurt O., Kaya E., Guven I. Comparación de la composición química y el potencial anti-metanogénico de las hojas de Liquidambar orientalis con hojas de Laurus nobilis y Eucalyptus globulus utilizando la técnica de producción de gas *in vitro*. Ciencia Invest Agraria 2017; 44(1): 75-82.

Joch M., Cermak L., Hakl J., Hucko B., Duskova D., Marounek M. *In vitro* screening of essential oil active compounds for manipulation of rumen fermentation and methane mitigation. Asian-Australasian Journal of Animal Sciences 2016: 29(7): 952.

Kara K. Nutrient matter, fatty acids, *in vitro* gas production and digestion of herbage and silage quality of yellow sweet clover (*Melilotus officinalis* L.) at different phenological stages. J Anim Feed Sci 2021; 30(2): 128-140.

Makkar HPS., Blümmel M., Becker K. *In vitro* rumen apparent and true digestibilities of tannin-rich forages. Anim Feed Sci Technology 1997; 67: 245-251.

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 2016; 55: 49-60.

Menke KH., Raab L., Salewski A., Steingass H., Fritz D., Schneider W. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. The J Agricultural Sci 1979; 93(1): 217-222.

Onel SE., Taylan A., Kanber 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 Üniversitesi Veteriner Fakültesi Dergisi 2020; 17(3): 283-289.

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; 78(12): 4271-4280.

Schultz MM., Neser FW., Makgahlela ML. A balanced perspective on the importance of extensive ruminant production for human nutrition and livelihoods and its contribution to greenhouse gas emissions. South African Journal of Science 2020; 116(9-10): 1-3.

Tural S., Turhan S. Antimicrobial and antioxidant properties of thyme (*Thymus vulgaris L.*), rosemary (*Rosmarinus officinalis L.*) and laurel (*Lauris nobilis L.*) essential oils and their mixtures. Gıda J of Food 2017; 42(5): 588-596.

Zhou R., Wu J., Lang X., Liu L., Casper DP., Wang C., Wei S. Effects of oregano essential oil on *in vitro* ruminal fermentation, methane production, and ruminal microbial community. J Dairy Sci 2020; 103(3): 2303-2314.