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The Effects of Laurel Volatile Oil (*Laurusnobilis L.)* on *In Vitro* Ruminal Gas Production of Methane Emission, Organic Acids and Protozoa Counts Alfalfa Herbage

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Summary: The aim of the present *in vitro* study was to investigate the effects of laurel volatile oil, (*Laurusnobilis L.)* on ruminal gas production, methane emission, organic acids and protozoa counts of Alfalfa herbage. The components of laurel volatile oil were analysed. The effects of the addition of laurel volatile oil, at levels of 0, 50, 100 and 200 mg/L (L0, L50, L100 and L200), to the rumen fluid on *in vitro*ruminal digestion were determined using by*in vitro* gas production technique. The addition of 50 mg/L of laurel volatile oil decreased total gas and methane production (ml), organic matter digestion (OMD), and metabolic energy (ME) values. On the other hand, the addition of this oil at levels of 100 mg/L and 200 mg/L was observed not to alter the *in vitro* total gas, methane (ml), ME and OMD values (P<0.05). While ruminal ammonia nitrogen levels decreased in Groups L50 and L100, no alteration was detected in Group L200 (P<0.05). Ruminal protozoa counts did not affected by the addition of laurel volatile oil within a range of 50-200 mg/L (P>0.05). The amounts of the total volatile fatty acids (TVFA) and butyric acid (BA) in the *in vitro* fermentation fluid of alfalfa herbage were low in all groups. It was determined that laurel volatile oil (*LaurusnobilisL.)* caused dose-dependent alterations in the *in vitro* digestion parameters.As a result, it has been determined that the active substances in Laurusnobilis L. essential oil may have regulation power on ruminal fermentation. It is thought that more research is needed to reveal the effects of Laurusnobilis L. volatile in terms of both ecological and digestive system physiology by using different feed types and essential oil combinations
Key words: *In vitro* gas production, laurel, methane, volatile oil

Yonca Kuru Otunun *İn Vitro* Ruminal Gaz Üretimi, Metan Salınımı, Organik Asit ve Protozoa Sayısı Üzerine Defne Uçucu Yağının (*Laurusnobilis* L.) Etkisi

Özet: Bu in vitro çalışmanın amacı, defne (*Laurusnobilis L.*) yapraklarından elde edilen defne uçucu yağının yonca kuru otunun ruminal gaz üretimini, metan emisyonunu, organik asitler ve protozoa üzerine etkilerini araştırmaktır. Defne uçucu yağının bileşenleri analiz edildi. Rumen sıvısına 0, 50, 100 ve 200 mg/L (L0, L50, L100 ve L200) seviyelerinde defne uçucu yağı eklenmesinin in vitro ruminal sindirime etkileri in vitro gaz üretimi ile belirlendi. 50 mg/L defne uçucu yağı ilavesi toplam gaz ve metan üretimini (ml), organik madde sindirimini (OMD) ve metabolik enerji (ME) değerlerini düşürmüştür. Öte yandan bu yağın 100 mg/L ve 200 mg / L seviyelerinde eklenmesinin in vitro toplam gaz, metan (ml), ME ve OMD değerlerini (P <0.05) değiştirmediği görülmüştür. Grup L50 ve L100'de ruminal amonyak nitrojen seviyeleri azalırken, Grup L200'de değişiklik saptanmadı (P <0.05). Ruminal protozoa sayıları, defne uçucu yağının 50-200 mg / L aralığında eklenmesinden etkilenmedi (P> 0.05). Yonca otunun in vitro fermentasyon sıvısındaki toplam uçucu yağ asitleri (TVFA) ve butirik asit (BA) miktarları tüm gruplarda düşüktü. Defne uçucu yağının (*Laurusnobilis L.*) in vitro sindirim parametrelerinde doza bağlı değişikliklere neden olduğu belirlendi. Sonuç olarak *Laurusnobilis L.* uçucu yağındaki etken maddelerin ruminal fermantasyon üzerinde düzenleme gücüne sahip olabileceği tespit edilmiştir. *Laurusnobilis L.* Uçucu yağının hem ekolojik hem de sindirim sistemi fizyolojisi açısından etkilerini farklı yem türleri ve uçucu yağ kombinasyonları kullanarak ortaya çıkarmak için daha fazla araştırmaya ihtiyaç olduğu düşünülmektedir.
Anahtar kelimeler: Defne, *in vitro* gaz üretimi, metan, uçucu yağ

Introduction

The consumption of animal products remains low in developing countries due to growing population. Therefore, the increasing demand for animal products points out to the need for researching how to increase animal production (Gemeda, 2018; Thornton and Pierre, 2010). Several studies have demonstrated that, on a global scale, 80 million tonnes of CH_4 is

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produced each year, nearly 47% of which originates from human activity on agricultural land and 39% from farm animals (Ellis et al., 2010; Gerber et al., 2013; Hook et al., 2010). Methane gas production by ruminants eventually results in a gross energy loss ranging from 2% to 12% (Wanapat et al., 2015). It has been reported that, depending on the type, particle size and dry matter content of the feed provided to them, cattle produce 60-160 kg CH_4/year per head, whilst sheep and goats produce 10-16 kg CH_4/year per head (Hristov et al., 2013). Methane is considered to be even more dangerous than carbon dioxide (CO_2), which also causes climate change and affects global warming (Bodas et al., 2012). Creating favourable environmental conditions that would reduce methane production is rather difficult. Various inhibition techniques have been used to reduce the production of enteric CH_4 (Patra et al., 2016). Unfortunately, some of these techniques have detrimental effects on ruminal microorganisms and fermentation (Patra and Yu, 2013). Furthermore, some CH_4 inhibitors are toxic for ruminants (Patra and Yu, 2012). Therefore, it is required to find safe alternative feed additives in order to protect the environment and sustain animal production while reducing enteric CH_4 emissions. In the past few years, consumers have called for the use of natural products, such as phytochemicals, to alter the ruminal ecosystem. In this context, the structures of the secondary metabolites of more than 200,000 plants have been determined (Hartmann, 2007).

The rich flora of Turkey contributes greatly to the abundance and diversity of plants from which volatile oils are derived. Apart from its geographical position and climate diversity, Turkey being situated at the crossroads of 3 very important floristic regions accounts for the diversity of aromatic herbs found throughout the country, as is the case with other plant species and genera. The aim of this study was to investigate the effects of laurel volatile oil, an export product (approx. 12,000 tonnes/year) of Turkey derived from the leaves of laurel trees native to the coastline of the country (Karık et al., 2015), on *in vitro* roughage degradability. Volatile oils show antimethanogenic effect on *in vitro* digestion and alter rumen fluid parameters (Cobellis et al., 2016; Ulger et al., 2017; Zhou et al., 2020). It is foreseen that, owing to the active substances it contains, laurel volatile oil may alter rumen fluid parameters and *in vitro* digestion. This study was aimed at determining the effects of different levels (0, 50, 100, 200 mg/L rumen fluid) of laurel volatile oil, an export product of Turkey (approx. 12,000 tonnes/year) derived from the leaves of laurel trees on the Mediterranean coastline, on *in vitro*ruminal gas production, methane emission, organic acids and protozoa counts.

Materials and Methods

Ethical approval

We obtained an approval (No: 2020/04-16) from the Local Ethics Committee of Hatay Mustafa Kemal University for this study.

Plant material

The laurel volatile oil used in this study was extracted by hydrodistillation from the leaves of laurel trees (*Laurusnobilis L.*) in the Hatay province of Turkey, which were collected at the blooming stage and dried at 35 °C.

Extraction and component analyses of laurel 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 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.

Chemical analysis of dried alfalfa

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

In vitro gas production

The *in vitro* degradability of dried alfalfa 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 fistulat-

ed 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. Laurel volatile oil (*Laurusnobilis L*.) was drawn into 100 ml-glass syringes (Model Fortuna, Germany) and added at levels of 50-100-200 mg/L to the ruminal fluid samples, and then incubated in 10 ml-aliquots with 200±10 mg of dried alfalfa 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 share 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 degradability parameters

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

$$ME\ (MJ\ /kg\ DM) = 2.20 + 0.136 \times GP + 0.057 \times CP + 0.0028597 \times EE^2$$

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

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 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 was filtered from each syringe and added 49 ml of a diluent (mixture of 20 ml of 37% formalin, 150 ml of glycerine and 820 ml of distilled water) to prepare ready-to-count 50-ml sample aliquots as described by Boyne et al. One ml of each aliquot was placed in the chamber of a Macmaster'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 17.0 software package. The statistical significance of the groups was determined by one-way analysis of variance (ANOVA). The dose-dependent differences of the *in vitro* digestion parameters were detected with polynomial contrast analysis (linear, quadratic and cubic). When statistical significance was detected, Tukey's

Table 1. Chemicalcomponents of *Laurusnobilis L*. volatileoil

Retention Time (RT)	Rate (%)	Components
12.90	5.18	α-pinene
13.08	0.47	α-thujene
17.51	4.73	β -pinene
18.53	7.57	sabinene
22.01	6.33	α-terpineol
24.43	42.82	1,8-cineole
26.41	0.66	γ -terpinene
28.14	2.34	p-cymene
28.83	0.22	terpinolene
40.72	1.01	trans-sabinenehydrate
45.91	3.18	linalool
47.18	0.22	pinocarvone
49.24	2.71	4-carvomenthenol
52.11	0.26	pinocarveol, trans
54.31	1.02	camphene
54.37	12.99	α-terpinylacetate
54.49	0.32	exo-norborneol
55.47	0.37	nerylacetate
65.05	0.55	caryophylleneoxide
65.66	1.56	methyleugenol
67.97	0.30	spathulenol
68.96	1.28	eugenol
69.13	0.47	α-elemene
70.07	0.31	α-eudesmol
70.32	0.79	β -eudesmol

multiple range test was performed as a multiple comparison test. The p-level for statistical significance was set below 0.05 (P<0.05).

Results

The dried alfalfa used in this study was determined to contain 94.38% dry matter, 7.13% crude ash, 3.4% crude fat and 20.44% crude protein. The chemical composition of the laurel volatile oil used in this study is presented in Table 1. The major components of this oil, in order of share, were determined as follows: 1,8-cineole (42.82%), α-terpinyl acetate (12.99%), sabinene (7.57%), α-terpineol (6.33%), and β-pinene (4.73%).

The effects of the different doses of laurel volatile oil (*Laurusnobilis L.*) on *in vitro* gas production, ammonia nitrogen level and protozoa count at the end of a 24 h-incubation period are shown in Table 2. The results obtained in the present study demonstrated that different doses of laurel volatile oil resulted in statistically significant differences. The first, second and third doses of laurel volatile oil were determined to have decreased gas production by rates of 20.8%, 7.3% and 7.4%, respectively (P<0.05).The addition of 50 mg/L of laurel volatile oil decreased total gas and methane production (ml), organic matter digestion (OMD), and metabolic energy (ME) values. On the other hand, the addition of this oil at levels of 100 mg/

Table 2. The effect of laurel volatile oil on the *in vitro* digestion parameters of alfalfa hay

	TGP (ml/0.2 g DM)	Methane (%)	Methane (ml)	ME (MJ/kg DM)	OMD (%)	NH_3-N (mg/dl)	Protozoa
L0 (control)	42.21±1.92^{a}	22.06±0.23^{c}	9.31±0.43^{ab}	8.98±0.26^{a}	67.02±1.70^{a}	47.82±0.23^{a}	2.72±0.10
L50	33.39±0.6^{b}	25.25±0.25^{ab}	8.43±0.22^{b}	7.78±0.08^{b}	59.18±0.53^{b}	43.79±1.84^{bc}	2.54±0.13
L100	39.11±1.17^{a}	24.70±0.46^{b}	9.66±0.37^{ab}	8.56±0.15^{a}	64.27±1.04^{a}	41.85±0.64^{c}	2.53±0.23
L200	39.06±1.39^{a}	26.77±0.41^{a}	10.45±0.37^{a}	8.55±0.19^{a}	64.22±1.24^{a}	45.75±1.93^{ab}	2.80±0.25
SEM	1.008	0.460	0.253	0.137	0.896	0.912	0.904
P-values							
L	NS	***	**	NS	NS	*	NS
Q	***	NS	*	***	***		
C	***	***	NS	***	***		

L0, L50, L100, L200: Groups added 0 (control), 50, 100, and 200 mg/L of laurel volatile oil, respectively.
TGP: Total gas production (24h ml/ 0.2 g DM), CH_4: methane production as a percentage of total gas production, ME: Metabolic energy as MJ/kg DM, OMD: Organic matter digestibility as %, NS: non-significant.
*Differences between the average values indicated with different letters in the same column are statistically significant (P<0.05).
** Differences between the average values indicated with different letters in the same column are statistically significant (P<0.01).
*** Differences between the average values indicated with different letters in the same column are statistically significant (P<0.001).

Table 3. The effect of laurel volatile oil (*Laurusnobilis L.*) on ruminal fermentation characteristics

	L0 (control)	L50	L100	L200	SEM	P value
TVFA	101.78±1.19^{a}	97.75±0.75^{c}	98.68±1.15^{b}	98.31±1.39^{d}	1.159	**
AA	52.65±0.97	51.20±0.96	52.38±0.79	51.03±0.67	0.427	NS
PA	24.85±1.08	20.81±1.26	23.70±0.61	21.21±0.51	0.601	NS
BA	17.59±0.29^{a}	13.98±0.65^{b}	13.87±0.61^{bc}	11.98±79^{c}	0.592	**
OFA	3.76	1.59	3.38	4.75	0.393	NS
AA/PA	2.11	2.46	2.23	2.41	0.227	NS

L50, L100, L200: Groups added 50, 100, and 200 mg/L of laurel 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; OFA: other fatty acids comprise of iso-butyrate + valerate + iso-valerate; AA: acetic acid, PA: propionic acid, BA: butyric acid, AA/PA: acetate / propionate
* Differences between the average values indicated with different letters in the same row are important
** Differences between the average values indicated with different letters in the same row are statistically significant (P<0.05).

L and 200 mg/L was observed not to alter the *in vitro* total gas, methane (ml), ME and OMD values (P<0.05). While ruminal ammonia nitrogen levels decreased in Groups L50 and L100, no alteration was detected in Group L200 (P<0.05). Ruminal protozoa counts were ascertained not to have been affected by the addition of laurel volatile oil within a range of 50-200 mg/L (P>0.05).

As shown in Table 3, the different doses of laurel volatile oil (*Laurusnobilis L*.) added to ruminal fluid significantly reduced the total volatile fatty acid (TVFA) and butyric acid levels (P<0.05). The dose most effective on ruminal fermentation was 50 mg/L.

Discussion and Conclusion

Volatile oil derived from the leaves of laurel trees, native to the Mediterranean coastline, is reported to have antibacterial effect, owing to the very high levels of 1, 8-cineole (42.82%), α-terpinyl acetate (12.99%), sabinene (7.57%), and α-terpineol (6.33%) it contains (Tural and Turhan, 2017). The volatile oil profile of laurel leaves from similar locations and the chemical composition of laurel volatile oil are to a great extent similar to those determined in the present study (Sangun et al., 2007; Ayanoğlu et al., 2010; Karık et al.,2015).

Gas production during *in vitro* incubation is generally considered to be a good indicator of ruminal degradability, fermentation and microbial activity, as higher gas levels indicate better nutrient sources for rumen microorganisms (Makkar et al., 1997). At the end of the study, it was determined that all of the laurel volatile oil (*Laurusnobilis L*.) levels tested had decreased gas production. Some researchers have reported increased gas production during *in vitro* incubation with other plant extracts rich in secondary metabolites (Jiménez-Peralta et al., 2011; Sallam et al., 2010).Limited data is available on the effect of laurel extract on *in vitro* ammonia nitrogen production, yet *in vivo* research has shown that, based on comparison with controls, ruminal NH_3-N and CH_4 levels significantly decrease with the use of volatile oils (Manh et al., 2012). In the present study, when compared to the control group, methane levels (ml) were determined to have significantly decreased in Group L50 (P<0.01). This result, which agrees with previous studies reporting reduced methane emissions with the use of volatile oils (Ravindra et al., 2009; Patra and Yu, 2012), was attributed to the high level of 8-cineole (42.82%) found in laurel leaves. Plant extracts and their administration doses should be selected in a way to avoid any negative effect on ruminal fermentation and feed durability. When used for dietary supplementation, volatile oils should induce a positive effect by reducing ammonia concentrations in the rumen. Although there is not enough literature on the effect of laurel essential oil on rumen parameters, the laurel volatile oil (*Laurusnobilis L*.) used in the present study was observed to have shown a positive effect by decreasing ruminal ammonia levels without affecting ruminal protozoa counts (Mandal et al., 2016; Onel and Aksu, 2017).

In the current study, the molarities of TVFA in rumen (98-102 mmol/L) in the fermentation process of alfalfa herbage was at an ideal level for the normal ruminal ecosystem (Kara et al., 2018). The fact that the laurel volatile oil does not have a negative effect on acetate and propionate levels indicates that it does not have a negative effect on digestibility. On the other hand, decreasing the butyric acid level of the herb doses decreased the TVFA molaritiesin fermentation fluid.

In view of digestibility, protozoal growth and reduced ammonia levels having been reported to be inhibited by the chemical composition of the plant (Tural and Turhan, 2017), the nitrogen-decreasing effect of laurel volatile oil (*Laurusnobilis L*.) was attributed to it most possibly inhibiting the growth of ammonia-producing bacteria. On the other hand, different from the results of *in vitro* research, several studies conducted in cattle and sheep have demonstrated that volatile oils do not affect bacterial nitrogen levels (Benchaar et al., 2006; Newbold et al., 2004). In result, it was determined that the addition of 50 mg/L of laurel volatile oil to the rumen fluid showed relatively adverse effects on the *in vitro*ruminal gas production of alfalfa herbage as well as on digestion parameters, yet also produced an antimethanogenic effect. There is a need for the positive effects of these plants and their extracts to be extended to *in vivo* conditions and to be tested in long-term studies.

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References

AOAC official methods of analysis (16th edn). AOAC International, Arlington, VA USA; 1995.

Ayanoğlu F, Mert A, Kaya A, Köse E. Hatay yöresinde doğal olarak yetişen defne (*Laurusnobilis L.*) bitkisinin kalite özelliklerinin belirlenmesi ve seleksiyonu, Tübitak Proje No: 108O878, 2010; p. 268.

Benchaar C, Petit HV, Berthiaume R, Whyte T D, Chouinard PY. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. J Dairy Sci 2006; 89(11): 4352-64.

Blümmel M, Makkar H PS, Becker K. In vitro gas production: A technique revisited. J Anim Phys

Anim Nutr 1997; 77(1): 24-34.

Bodas R, Prieto N, García-González R, Andrés S, Giráldez FJ, López S. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim Feed Sci Technology 2012; 176(1-4): 78-93.

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

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 Technology 2016; 215: 25-36.

Ellis JL, Bannink A, France J, Kebreab E, Dijkstra J. Evaluation of enteric methane prediction equations for dairy cows used in whole farm models. Glob Chang Biol 2010; 16(12):3246-56.

Gemeda BS. The potential of tropical tannin rich browses in reduction of enteric methane. Appro Poult Dairy Vet Sci 2018; 2(3):154-62.

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): 220-34.

Hartmann T. From waste products to ecochemicals: fifty years research of plant secondary metabolism. Phytochemistry 2007; 68(22-24): 2831-46.

Hook SE, André Denis GW, Brian WM. Methanogens: Methane producers of the rumen and mitigation strategies. Archaea 2010; ID 945785: doi:10.1155/2010/945785.

Hristov AN, Oh J, Firkins JL, Dijkstra J, Kebreab E, Waghorn G, Makkar HPS, Adesogan AT, Yang W, Lee C, Gerber PJ, Henderson B, Tricarico JM. Specıal topıcs-mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J Anim Sci2013; 91(11): 5045-69.

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.

Jiménez-Peralta FS, Salem AZM, Mejia-Hernández P, González-Ronquillo M, Albarrán-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. Livestock Sci 2011; 136(2-3): 192-200.

Kara K, Ozkaya S, Baytok E, Guclu BK, Aktug E, Erbas S. Effect of phenological stage on nutrient composition, *in vitro* fermentation and gas production kinetics of *Plantago lanceolata* herbage. Vet Med 2018; 63: 251-60.

Karık Ü, Çiçek F, Tutar M, Ayas F. Türkiye defne (*Laurus nobilis L.*) populasyonlarının uçucu yağ bileşenleri. Anadolu J of Aari 2015; 25(1): 1-16.

Makkar HPS, Blümmel M, Becker K. In vitro rumen apparent and true digestibilities of tannin-rich forages. Anim Feed Sci Technology 1997; 67: 245-51.

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. Research Article Indian J Anim 2016; 55: 49-60.

Manh NS, Wanapat M, Uriyapongson S, Khejornsart P, Chanthakhoun V. Effect of eucalyptus (*Camaldulensis*) leaf meal powder on rumen fermentation characteristics in cattle fed on rice straw. Afr J Agric Res 2012; 7(14): 2142-8.

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

Newbold CJ, McIntosh FM, Williams P, Riccardo L, Wallace RJ. Effects of a specific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci Techn 2004; 114(1-4): 105-12.

Onel SE, Aksu T. Esansiyel / uçucu yağlar: Esansiyel / uçucu yağların hayvan beslemede kullanımı. Türkiye Klinikleri Hay Bes ve Besl Has-Özel Konular 2017; 3(1): 21-9.

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; 78(12): 4271-80.

Patra AK, Yu Z. Effective reduction of enteric methane production by a combination of nitrate and saponin without adverse effect on feed degradability, fermentation, or bacterial and archaeal communities of the rumen. Bioresource Techn 2013; 148: 352-60.

Patra PK, Saeki T, Edward JD, Ishijima K, Umezawa T, Ito A, Aoki S, Morimoto S, Kort EA, Crotwell A, Kumar KR, Nakazawa T. Regional methane emission estimation based on observed atmospheric concentrations (2002-2012). J Meteor Soc Japan 2016; 94(1): 91-113.

Ravindra K, Kamra DN, Neeta A, Chaudhary LC. Effect of eucalyptus (*Eucalyptus globulus*) oil on in vitro methanogenesis and fermentation of feed with buffalo rumen liquor. Anim Nutr Feed Techn 2009; 9: 237-43.

Sallam SM, Bueno IC, Nasser ME, Abdalla AL. Effect of eucalyptus (*Eucalyptus citriodora*) fresh or residue leaves on methane emission in vitro. Italian J Anim Sci 2010; 9(3): 58.

Sangun MK, Aydin E, Timur M, Karadeniz H, Caliskan M, Ozkan A. Comparison of chemical composition of the essential oil of laurus nobilis l. leaves and fruits from different regions of Hatay, Turkey. J Environ Biol 2007; 28(4):731-3.

Thornto PK, Pierre JG. Climate change and the growth of the livestock sector in developing countries. Mitigation Adapt Strateg Glob Change 2010; 15(2):169-84.

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. Gida J of Food 2017; 42(5): 588-96.

Van Soest PV, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991; 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; 1(3): 96-103.

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): 2303-14.