JOURNAL OF AGRICULTURE

RESEARCH ARTICLE

ISSN: 2636-8757

Assessing the Effects of Clove Oil on Gas and Methane Production of Some Roughages Using *In Vitro* Gas Production Technique

Emrah KAYA*

Iğdır University, Faculty of Agriculture, Department of Animal Science, Iğdır, Turkey.

Correspondence
Emrah KAYA, Iğdır University, Faculty of Agriculture, Department of Animal Science, Iğdır, Turkey.
Email:emrah.kaya@igdir.edu.tr

This study does not required ethics comitee approval.

Abstract

This study aimed to examine the effects of fermentation parameters on adding clove oil to oat straw, alfalfa hay, and maize silage at a 3% dry matter basis. *In vitro* gas production, methane production (CH_4), metabolic energy (ME), net energy lactation (NE_L), and organic matter digestion (OMD) were all influenced by the addition of clove oil to oat straw, alfalfa hay, and maize silage (P<0.001). The *in vitro* gas production, CH_4 (mL), CH_4 (%), ME, NE_L, and OMD of roughages were found to be between 29.48-48.31 mL, 4.95-8.37 mL, 15.11-19.96%, 6.53-9.68 MJ kg^{-1} DM, 3.59-5.79 MJ kg^{-1} DM, and 41.03-57.64%, respectively. When roughage samples with and without addition were compared, clove oil supplementation decreased net gas production values by 10 to 28% (P<0.001). The CH_4 of roughages with and without clove oil was examined. A reduction of 1.31, 2.67, and 3.12 was observed on a mL basis, to 11.64, 12.17, and 15.23% on a % methane basis, respectively. At ME, NE_L, and OMD values calculated with gas production values and chemical composition data, the lowest values were found in oat straw with clove oil added, and the highest in alfalfa hay without additions (P<0.001).
As a consequence, the gas production values of the roughages decreased significantly with the addition of clove oil. However, the decrease had no anti-methanogenic effect on CH_4, one of the greenhouse gases released by ruminants. The use of clove oil in addition to roughage to decrease gas and methane production may be advised, although the effect on total mixed rations or animals should be examined further through *in situ* and *in vivo* investigations.

Key words: Clove oil, gas production, *in vitro*, methane, roughage.

1. INTRODUCTION

It is known that antibiotic feed additives are utilized in ruminant animals to increase feed efficiency to prevent metabolic illnesses and diseases and to reduce the amount of methane gas produced in the environment, and it is still being researched (Gerber et al., 2013; Jouany and Morgavi, 2007). Antibiotic feed additives have been banned in the European Union since January 2006 because of transmission from animal products to people, causing antibiotic resistance in microorganisms (Chesson, 2006). The interest in alternative plant-based feed additives that increase the performance of animals instead of antibiotics and are acceptable in terms of environmental friendliness has led researchers to work in this direction (Sarnataro et al., 2020; Geraci et al., 2012; Busquet et al., 2006; Patra et al., 2006). Essential oils are plant secondary metabolites composed of terpenoids and phenylpropanoids isolated from various plant sections (Calsamiglia et al., 2007). Essential oils have been shown to have the ability to reduce energy loss and greenhouse gas emissions through CH_4 gas (Benchaar and Greathead, 2011; Nanon et al. 2015; Ratika and Singh, 2018). They have also been shown to inhibit rumen microbes, total volatile fatty acids, total gas generation, and feed digestion (Wallace et al., 2002; Castillejos et al., 2006; Joch et al., 2019). It has been reported that clove oil is the active component of Eugenol and has appetizing, digestive stimulant, and antiseptic properties (Frankli̇č et. al., 2009). Also, it contains 14.71% Caryophyllene, 14.13% Eugenyl acetate, and 66.59% Eugenol in its chemical composition (Özüretmen,

Received: 30-09-2022 | Accepted: 21-11-2022

2013). Oils as a feed addition have been used as an option to minimize CH_4 in the rumen in recent years (Boadi et al., 2004; Martin et al., 2010). Methane, a byproduct of ruminal microbial fermentation, contributes significantly to global warming (IPCC, 2014). Ruminants are crucial participants in the production of CH_4. The quantity of CH_4 produced in the rumen is used to calculate environmental consequences and energy costs in the animal production industry (Auffret et al., 2018). It is estimated that 2-15% of feed energy is wasted as CH_4 throughout rumen fermentation. Furthermore, the greenhouse impact of CH_4 is 23 times that of CO_2 (Kim et al. 2012). Dong et al. (2010) concluded that the addition of various plant extracts to different diets reduced CH_4 emissions. According to Martin et al. (2010), the most efficient approach to reducing methane emissions is to enhance ruminant diets with oil. Essential oils and their constituents have been shown to influence rumen fermentation and increase nutrient use in ruminants (Cardoza et al., 2004; Busquet et al., 2006; Calsamiglia et al., 2007). Ruminant nutrition consists of a variety of vegetables, animal by products, and oils (NRC, 2001). Several dietary and nutritional techniques, such as using essential oils in the feed, have been suggested to reduce ruminant enteric CH_4 emissions (Benchaar and Greathead, 2011). It is also stated that farm animals contribute 18% of greenhouse gas emissions. It was estimated that around 15% of this constituent was produced by fermentation in rumens and ruminant animal manure (Takahashi et al., 2005). The addition of oil to animal feeds has been reported *in vivo* and *in vitro* studies to kill pathogenic microorganisms in the digestive system, improve the flavor and consumability of the feed, increase digestibility, improve feed efficiency, increase weight gain, and contribute to a healthier environment by forming compounds with ammonia (Tuncer et al., 1989; Kutlu and Görgülü, 2001) The *in vitro* gas production methodology can determine the potential degradability and degradation rate of feeds. This study examined the effects of fermentation parameters on the addition of clove oil to oat straw, alfalfa hay, and maize silage at 3% dry matter.

2. MATERIALS AND METHODS

In the study, oat straw, alfalfa hay, and maize silage (Table 1), which are used in dairy cattle rations in the Atatürk University Research and Application Center, were used as feed material. Experimental groups were prepared by adding 3% clove oil to raughages. Clove oil, used as an additive, was obtained from a commercial herbalist from Iğdır Province, Turkey. The research was carried out at Atatürk University's Laboratory of Feed and Animal Nutrition, Department of Animal Science, Faculty of Agriculture, Erzurum, Turkey.

The roughage samples were dried at 70°C for 72 hours in an oven until they reached a consistent weight, then powdered in a grinder to pass a 1 mm filter and stored for further nutrient content and gas production (GP) parameters assessment..

Table 1. *Nutrient content of roughages*

	DM (%)	CA (%)	EE (%)	CP (%)	ADF (%)	NDF (%)
Oat straw	94.92	6.39	1.20	4.94	39.05	65.98
Alfalfa hay	95.04	6.97	1.97	16.05	38.94	56.78
Maize silage	93.28	8.84	2.52	9.85	34.02	67.05

DM: Dry matter (%), CA: Crude ash (% of DM), EE: Ether extract (% of DM), CP: Crude protein (% of DM), ADF: Acid detergent fiber (% of DM), NDF: Neutral detergent fiber (% of DM)

The DM, CA, EE, and CP analyses of roughages were determined with the methods reported by AOAC (1990); the ADF and NDF were obtained using the ANKOM and the procedures described by Van Soest et al. (1991).

The amount of gas released as a result of the 24-hour incubation of the ration by method Menke et al. (1979) according to the technique reported. The rumen fluid used in the analysis was obtained from two cattle brought to the slaughterhouse for slaughter. The methane contents produced at the end of the 24-hour incubation were determined using an infrared methane analyzer (Goel et al., 2008).

The following equation was used to determine the methane production of roughages per mL;

$$CH_4 \text{ production (mL)} = \text{Total gas production (mL)} \times \text{Percentage of } CH_4 \text{ (\%)}$$

The ME, NE_L, and OMD values of the roughages were calculated with the following equations stated by Menke and Steingass (1988). Data from GP, CP, EE, and CA in the equations was used.

$$ME = 2.2 + 0.1357 \times GP + 0.057 \times CP + 0.002859 \times EE^2 \quad (1)$$

$$NE_L = 0.101 \times GP + 0.051 \times CP + 0.112 \times EE \quad (2)$$

$$OMD = 14.88 + 0.8893 \times GP + 0.448 \times CP + 0.651 \times CA \quad (3)$$

ME: Metabolisable energy (MJ Kg^{-1} DM) GP: Gas production of 24H (200 mg mL^{-1})
CP: Crude protein (%) EE: Ether extract (%) NE_L: Net energy for lactation (MJ Kg^{-1} DM)
OMD: Organic matter digestibility (%) CA: Crude ash (%)

One-way analysis of variance (ANOVA) was used to determine the effects of clove oil on gas production, methane production, metabolisable energy, and organic matter digestibility of the roughages. The obtained data were analyzed using SPSS 17.0 (2011), and the differences between the means were determined using Tukey multiple comparison tests. The mean differences were considered significant at $p<0.001$.

3. RESULTS AND DISCUSSION

The effects of clove oil on gas production, methane production, metabolisable energy and organic matter digestibility were given in Table 2. *In vitro* gas production, methane production (CH_4), metabolic energy (ME), net energy lactation (NE_L), and organic matter digestion (OMD) were all influenced by the addition of clove oil to oat straw, alfalfa hay, and maize silage (P<0.001). The *in vitro* gas production, CH_4 (mL), CH_4 (%), ME, NE_L, and OMD of roughages were found to be between 29.48-48.31 mL, 4.95-8.37 mL, 15.11-19.96%, 6.53-9.68 MJ kg^{-1} DM, 3.59-5.79 MJ kg^{-1} DM, and 41.03-57.64%, respectively.

Table 2. *Gas production, methane content, metabolic energy, net energy for lactation and organic matter digestibility of roughages and clove oil mixtures*

	Net Gas (mL)	CH_4 (mL)	CH_4 (%)	ME (MJ Kg^{-1} DM)	NE_L (MJ Kg^{-1} DM)	OMD (%)
Oat straw	38.37^{b}	7.66^{a}	19.96^{a}	7.69^{b}	4.41^{b}	48.54^{b}
Alfalfa hay	48.31^{a}	8.37^{a}	17.34^{b}	9.68^{a}	5.79^{a}	57.64^{a}
Maize silage	36.59^{bc}	6.26^{b}	17.10^{b}	7.75^{b}	4.43^{b}	47.49^{b}
Oat straw + %3 CO	29.48^{d}	4.99^{c}	16.92^{b}	6.53^{c}	3.59^{c}	41.03^{c}
Alfalfa hay + %3 CO	34.46^{bcd}	5.25^{bc}	15.23^{c}	7.86^{b}	4.50^{b}	45.93^{b}
Maize silage + %3 CO	32.68^{cd}	4.95^{c}	15.11^{c}	7.28^{bc}	4.10^{bc}	44.19^{bc}
SEM	1.69	0.33	0.29	0.23	0.16	1.43
P.	0.000	0.000	0.000	0.000	0.000	0.000

[abc]Column means with common superscripts do not differ. CH_4: Methane, ME: Metabolisable Energy (MJ kg^{-1} DM), NE_L: Net Energy for Lactation (MJ kg^{-1} DM), OMD: Organic Matter Digestibility (%), CO: Clove Oil, SEM: Standard Error Mean, Sig: Significant level, ***: P<0.001

Comparing the roughage samples with and without additives, the addition of clove oil reduced the net gas production values by 10 to 28% (P<0.001). The CH_4 of roughages with and without clove oil was examined. A reduction of 1.31, 2.67, and 3.12 was observed on an mL basis, which corresponds to 11.64, 12.17, and 15.23% on a % methane basis, respectively. Sembiring and Baba (2022) tested the *in vitro* gas production of different roughages and reported that the gas productions of between 44 and 68 mL in 24 hours. The stated values are higher than the without additive roughage values obtained in the current study. With the addition of clove oil, the gas values obtained from the present study decreased further. Kaya and Kaya (2021) reported that decreasing in methane percentage (1.2%) with adding corn oil to TMR compared to the control group. Although the stated value is very low compared to the current study, the effect of feed additives in TMR on gas production values of feeds needs to be tested with further studies.

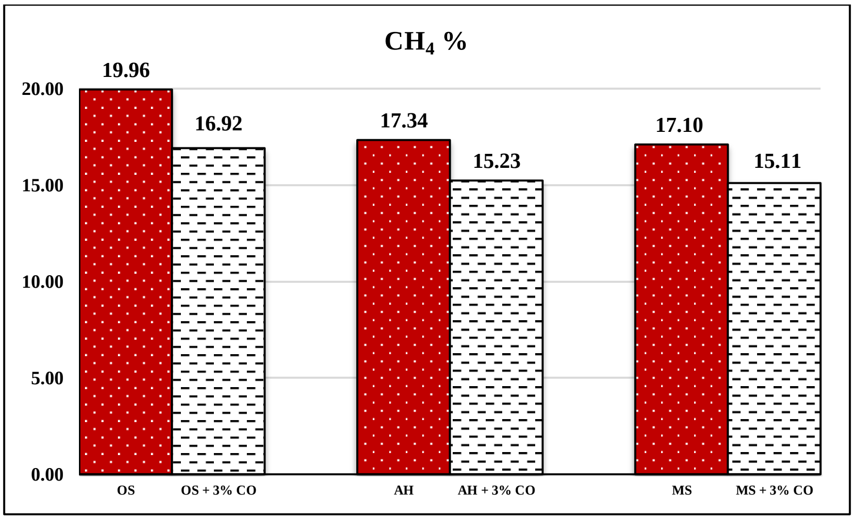

Figure 1. *Percentage methane changes of forages with and without clove oil addition*

Baraka and Abdl-Rahman (2012) investigated the effects of fumaric acid (0.5 mg L^{-1}) and different doses of eugenol (100, 200, and 400 mg L^{-1}) combinations on *in vitro* rumen fermentation. It was observed that the total VFA ratio and the amount of CH_4 gas decreased in the combination. The stated results are consistent with the data obtained as a result of the current study. The fact that eugenol, the active ingredient of clove oil, has digestive stimulant and antiseptic properties, is thought to be related to the results of the study.

At ME, NE_L, and OMD values calculated with gas production values and chemical composition data, the lowest values were found in oat straw with clove oil added, and the highest in alfalfa hay without additions (P<0.001).

Canbolat et al. (2011) investigated the effects of some essential oils (thyme, clove, mint, orange, and cinnamon) on *in vitro* gas production, OMD, and rumen fermentation of sainfoin, another roughage. It has been reported that with the addition of essential oils OMD and ME, a decrease was observed in the production of VFA, NH_3-N, and CH_4 gas. In the study, OMD and ME values for the control group were 69.7% and 10.8 MJ kg^{-1}, respectively, while in terms of the same parameters, they were found to be 55.7% and 8.6 MJ kg^{-1} for clove oil. The stated ME and OMD values are higher than the values obtained in the current study. Since the chemical composition of the feeds and gas production values are used in the calculation of the ME and OMD values, it is thought that the roughage types may have an effect on the variability of the current values.

In a study on *in vitro* dry matter digestibility and ME value of clove essential oil, it was reported that a dose of 300 ppm increased feed digestibility (10%) and ME value (11%) (Rofiq et al., 2012). The reported results are conflict with the data obtained as a result of the present study.

It has also been reported that essential oils have different effects on rumen bacteria; they stimulate microbial activity in some cases, but generally negatively affect the digestion of feed and reduce the metabolizable energy (ME) value (Canbolat et al., 2010).

According to Lopez et al. (2010), methane gas levels generated in feeds may be classified as low anti-mehanogenic (>11% and 14%), medium anti-metanogenic (>6% and 11%), or high anti-methanogenic (>0% and 6%). By taking these classes into account, ruminant energy usage efficiency may be raised while methane gas, which causes global warming, can be minimized. The result of this study indicate that the addition of clove oil decreases the methane percentages of roughages to some extent, but not sufficiently to be classified as anti-methanogenic.

4. CONCLUSION

Considering the results of many studies in the field of animal nutrition, it is seen that oils obtained from different plants have an effect on enteric gas production. However, the desired results could not be achieved in terms of minimizing the environmental damage to the emissions in terms of methane percentage.

As a consequence, the gas production values of the roughages decreased significantly with the addition of clove oil. However, the reduction had no anti-methanogenic effect on CH_4, one of the greenhouse gases released by ruminants. The use of clove oil in addition to roughage may be advised to decrease gas and methane production, but the effect on total mixed rations or animals should be examined further studies through *in situ* and *in vivo* investigations.

5. ACKNOWLEDGEMENTS

The author would like to thank to Assoc. Prof. Dr. Adem KAYA and Res. Asst. Ali KAYA from Atatürk University for their help in use of laboratory facilities.

6. AUTHOR CONTRIBUTIONS

The authors declare that they have contributed equally to the article.

7. CONFLICT OF INTEREST

The author declares no conflict of interest.

8. REFERENCES

AOAC., (1990). Official method of analysis. 15th ed., Association of Official Analytical Chemists, Washington, DC, USA. pp.66-88.

Auffret, M.D., Stewart, R., Dewhurst, R.J., Duthie, C.A., Rooke, J.A., Wallace, R.J., Freeman, T.C., Snelling, T.J., Watson, M., Roehe, R. (2018). Identification, comparison, and validation of robust rumen microbial biomarkers for methane emissions using diverse Bos Taurus breeds and basal diets. Front. Microb, 8(2), 36-42.

Benchaar, C., Greathead, H. (2011). Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Animal Feed Science and Technology, 166-167: 338-355.

Boadi, D., Benchaar, C., Chiquette, J., Masse, D. (2004). Mitigation strategies to reduce enteric methane emissions from dairy cows: update review, Canadian Journal of Animal Science, 84, 319-335.

Busquet, M., Calsamiglia, S., Ferret, A., Kamel, C. (2006). Plant extracts affect *in vitro* rumen microbial fermentation. Journal of dairy science, 89(2), 761-771.

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., Ferret, A. (2007). Invited review: essential oils as modifiers of rumen microbial fermentation, J. Dairy Sci. 90: 2580–2595 pp.

Canbolat, Ö., Kalkan, H., Karaman, Ş., Filya, İ. (2011). Esansiyel yağların sindirim, rumen fermantesyonu ve mikrobiyal protein üretimi üzerine etkileri, Kafkas Üniv Vet Fak Derg. 17 (4), 557–565.

Canbolat, Ö., Karaman, Ş., Filya, İ. (2010), Farklı kekik yağı dozlarının mısır silajının sindirimi ve rumen fermentasyonu üzerine etkileri, Kafkas Univ Vet Fak Derg., 16 (6): 933-939.

Cardoza, P.W., Calsamiglia, S., Ferret, A., Camel, C. (2004). Effect of natural plant extracts on ruminal protein degration and profiles in fermentation continuous culture. Journal of Animal Science. 82, 3230-3236.

Castillejos, L., Calsamiglia, S., Ferret, A. (2006). Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in *in vitro* systems. Journal of Dairy Science, 89: 2649-2658.

Chesson, A. (2006). Phasing out antibiotic feed additives in the EU: Worldwide relevance for animal food production. In: Barug D, de Jong J, Kies AK, Verstegen MWA, eds. Antimicrobial Growth Promoters: Where Do We Go from Here?. The Netherlands: Wageningen Academic Publishers, pp. 69-81.

Dong, G.Z., Wang, X.J., Liu, Z.B., Wang, F. (2010). Effects of phytogenic products on *in vitro* rumen fermentation and methane emission in goats. J. Anim. Feed Sci. 19, 218-229.

Franlič, T., Voljč, M., Salobir, J., Rezar, V. (2009). Use of herbs and spices and their extracts in animal nutrition. Acta Argiculturae Slovenica, 2 (94): 95–102pp.

Geraci, J.I., Garciarena, A.D., Gagliostro, G.A., Beauchemin, K.A., Colombatto, D. (2012). Plant extracts containing cinnamaldehyde, eugenol and capsicum oleoresin added to feedlot cattle diets: Ruminal environment, short term intake pattern and animal performance. Animal feed science and technology, 176(1-4), 123-130.

Gerber, P.J., Hristov, A.N., Henderson, B., Makkar, H., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan, A.T., Yang, W.Z., Tricarico, M., Kebreab, E., Waghorn, G., Dijkstra, J., Oosting, S. (2013). Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review. Animal, 7(s2), 220-234.

Goel, G., Makkar, H.P.S., Becker, K. (2008). Effect of sesbania sesban and carduus pycnocephalus leaves and fenugreek (Trigonella foenum-graecum L) seeds and their extract on partitioning of nutrients from roughage-and concentrate-based feeds to methane. Animal Feed Science and Technology, 147(1-3), 72-89.

IPCC, Field, C.B. (Ed.). (2014). Climate change 2014–Impacts, adaptation and vulnerability: Regional aspects. Cambridge University Press.

Joch, M.V., Kudrnaa, V., Haklc, J., Bo žikd, M., Homolkaa, P., Illeka, J., Tyrolováa, T., Výbornáa, A. (2019). *In vitro* and *in vivo* potential of a blend of essential oil compounds to improve rumen fermentation and performance of dairy cows. Animal Feed Science and Technology, 251, 176-186.

Jouany, J.P., Morgavi, D.P. (2007). Use of ‘natural’products as alternatives to antibiotic feed additives in ruminant production. Animal, 1(10), 1443-1466.

Kaya, A., Kaya, A. (2021). The effect of some vegetable oils added to dairy calf rations on *in vitro* feed value and enteric methane production. Journal of Agricultural Production, 2(1), 1-6.

Kim, E.T., Kim, C., Min, K., Lee, S.S. (2012). Effects of plant extracts on microbial population, methane emission and ruminal fermentation characteristics *in vitro*. Asian-Australasian Journal of Animal Sciences 25(6): 806-811.

Kutlu, H.R., Görgülü, M. (2001). Kanatlı yemlerinde yem katkı maddesi olarak kullanılan antibiyotik-büyütme faktörü için alternatifler. Yem Magazin Derg, 27, 45-62.

Martin, C., Morgavi, D., Doreau, M. (2010). Methane mitigation in ruminants: from microbe to the farm scale. Animal, 4(3), 351-365.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W. (1979). 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*. Journal of Agricultural Science, 93, 217-222.

Menke, K.H., Steingass, H. (1988). Estimation of energetic feed value obtained from chemical analysis and *in vitro* gas production. Animal Research Development, 28, 7-55.

Nanon, A, Suksombat, W., Yang, W.Z. (2015). Use of essential oils for manipulation of rumen microbial fermentation using batch culture. Thai Journal of Veterinary Medicine 45(2): 167-180.

NRC. (2001). Nutrient requirements of dairy cattle, 7th revised edition. Washington (DC): National Academy Press.

Patra, A.K., Kamra, D.N., Agarwal, N. (2006). Effect of plant extracts on *in vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Animal Feed Science and Technology, 128(3-4), 276-291.

Rofiq, M.N., Görgülü, M., Boğa, M. (2012). Karanfil uçucu yağının (Clove Oil) ruminantlarda *in vitro* gerçek KM ve NDF sindirilebilirliği ve yemin enerji içeriğine etkileri, 8. Ulusal Zootekni Öğrenci Kongresi, 127-129s.

Ratika, K., Singh, R.K.J. (2018). Plant derived essential oil in ruminant nutrition - A Review. International Journal of Current Microbiology and Applied Sciences 7(5): 1747-1753.

Sarnataro, C., Spanghero, M., Lavrenčič, A. (2020). Supplementation of diets with tannins from Chestnut wood or an extract from Stevia rebaudiana Bertoni and effects on *in vitro* rumen fermentation, protozoa count and methane production. Journal of Animal Physiology and Animal Nutrition, 104(5), 1310-1316.

Sembiring, M., Baba, A.S.H. (2022). *In Vitro* Gas Production Test: Towards rapid nutritional evaluation of roughages for ruminant feeding. Formosa Journal of Science and Technology (FJST) Vol. 1, No. 4, 2022: 247-258.

SPSS, (2011). IBM SPSS statistics for Windows, version 17.0. New York: IBM Corp 440.

Takahashi, J., Mwenya, B., Santoso, B., Sar, C., Umetsu, K., Kishimoto, T., Nishizaki, K., Kimura, K., Hamamoto, O. (2005). Mitigation of methane emission and energy recycling in animal agricultural systems. Asian-Australasian Journal of Animal Science 18(8): 1199-1208.

Tuncer, Ş.D., Kocabatmaz, M., Coşkun, B., Şeker, E. (1989). Kimyasal maddelerle muamele edilen arpa samanının sindirilme derecesinin naylon kese (nylon bag) tekniği ile tespit edilmesi. Doğa Türk Vet. Ve Hay. Derg., 13 (1): 66-81.

Üretmen, S. (2013). Buğday samanının karanfil eterik yağı ile muamelesinin *in vitro* organik madde sindirilebilirliği ve metabolize olabilir enerji değeri üzerine etkisi. Ege Üniversitesi Fen Bilimleri Enstitüsü Yüksek Lisans Tezi. Bornova-İzmir ss: 61.

Wallace, R.J., McEwan, N.R., McIntosh, F.M., Teferedegne, B., Newbold, C.J. (2002). Natural products as manipulators of rumen fermentation. Asian-Australasian Journal of Animal Sciences 15, 1458-1468.

Van Soest, P.V., Robertson, J.B., Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74(10), 3583- 3597.