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Determination of Nutritional Value and Methane Production Potential of *Phacelia tanacetifolia* in Different Stages of Growth

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ABSTRACT

Backround: This study was carried out to determine the nutritional value and methane production potential of *Phacelia tanacetifolia* plant in different growth stages in April-May year in the ecological conditions of the province of Adıyaman in Turkey.

Methods: Phacelia (*Phacelia tanacetifolia*) plant samples were collected with four replications in four different stages, pre-flowering, early flowering, mid-flowering and end of flowering (seeding). The nutrients of the feed samples were determined. The gas production was measured after 24 hours of incubation of the forage with rumen fluid.

Result: The effect of harvest time on chemical composition was highly significant. *In vitro* gas production was lowest in stage I. and highest in II. The methane (CH_4) lowest production values were found in the 1st stage, whereas the highest value was observed in the 4th stage (seed-binding stage), where the difference was statistically significant. ME was 7.49, 9.54, 8.90 and 8.61 MJ/kg DM respectively and the difference between the stages was statistically significant. It was concluded that *Phacelia tanacetifolia*, which is known as an important nectar source in the world and in Turkey. It is appropriate to harvest the phacelia plants in the second stage, *i.e.*, at early flowering for higher ME contents and OMD.

Key words: Digestibility, *in Vitro*, Nutrient content, Optimum Harvesting stage.

INTRODUCTION

In ruminant nutrition, feed costs account for 60-70% of production costs. In order to ensure the sustainability of livestock production and to obtain high quality and economical products, rations for ruminants must contain significant amounts of coarse forage. Turkish meadows and pastures are of great importance for coarse forage production in the country. Livestock production in Turkey depends on natural pastures. In recent years, indoor livestock production has gained importance. The productivity of meadows and pastures, which provide a large part of the fodder needs of Turkish livestock, has declined due to centuries of irresponsible use and high livestock numbers. For this reason, part of the agricultural land should be reserved for growing fodder crops as part of crop rotations. A plant suitable for this purpose is phacelia (*Phacelia tanacetifolia*). Phacelia is also known as a bee plant, as it is one of the rare plants that can meet roughage needs and provided a source of pollen and nectar for bees in early spring. Studies conducted in Turkey and other countries have shown that Phacelia can be grown both as a forage crop and as an important nectar source. As a forage crop, Phacelia can be fed to animals in the form of green and dry grass and for this purpose, it is beneficial to harvest it preferabily during the 50% flowering stagestage. Since it is used as a source of nectar during the flowering stagestage, it loses this characteristic towards the end of the flowering stagestage.

Although phacelia loses quality, harvesting the plant at the end of the flowering stagestage provides the opportunity to use the same area as bee pasture and forage crop (Sağlamtimur *et al*., 1989). Climate change and global warming are known to be a major problem that can endanger the life of living beings in the world (Sağlam *et al*., 2008). Greenhouse gases (CH_4, N_2O), which come from agriculture and livestock in all countries, cause global warming. Methane gas released to nature from dairy farms in the United States accounts for about 71% of methane emissions from agricultural activities and 19% of total methane emissions (Steinfeld *et al*., 2006;). Manure from ruminants accounts for 16.4% of annual methane gas production. This accounts for about 2.9% of all greenhouse gases causing global warming (Johnson *et al*., 1991). In studies to reduce methane emissions in ruminants, remarkable results have been obtained by using plant-based feed additives (Wallace, 2004). Essential oils of plants such as onion, ginger, clove, fennel and garlic have been found to reduce methane production under *in vitro* conditions (Kamra *et al*., 2006).

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In this study, in addition to the nutritional value of grass samples collected during certain growing seasons of the plant *Phacelia tanacetifolia*, its effect on methane gas causing global warming and energy losses, especially in ruminants, was investigated.

MATERIALS AND METHODS

The forage material used in this study was obtained from *Phacelia* plants by mowing at 15-day intervals in April-May 2016 in different regions under the ecological conditions of Adıyaman Province, Turkey and at the site of the Provincial Directorate of Food, Agriculture and Livestock of Adıyaman (37.77 latitude and 38.26 longitude). Plant samples of Phacelia were collected with four replicates at four different times, pre-flowering, early flowering, mid flowering and end of flowering (seeding) and the dry matter content was determined by taking the samples to the laboratory. A sufficient amount of rumen fluid was collected from yearling lambs slaughtered in slaughter houses and placed in a previously prepared hot water bath, with the addition of CO_2 to ensure that anaerobic conditions prevailed. The dried leaves of the Phacelia plant were ground so that they pass through a 1-mm sieve and prepared for chemical analysis. Crude nutrients of the forage samples used in the study (dry matter (DM), crude protein (CP), crude fat (CF), crude ash (CA)) were determined according to the standards given by AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF), which are the cell wall components of the forage, were determined according to the method described (Van Soest *et al*., 1991; Van Soest, 1994). The condensed tannin content of Phacelia leaves was determined according to the method described by Makkar *et al*., (1995). The organic matter digestion degree (OMDD) and metabolic energy (ME) values of Phacelia leaves were calculated using an equation described previously by Makkar *et al*., (1995). In this study, the method described by Makkar *et al*. was used to calculate gas production (Menke *et al*., 1979). This method is based on the calculation of the amount of gas formed after 24 hours of incubation of the forage with rumen fluid. The methane concentration (%) of the gasses formed after 24 hours of incubation was determined using an infrared gas analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel *et al*., 2008).

ME (MJ/kg DM) and OMD of phacelia plants were estimated using the equation of Menke and Steingass (1988) as follows:

ME (MJ/kg DM)=

1.68 + 0.1418GP + 0.073CP + 0.217EE- 0.028CA 1

OMD (%)= 14.88 + 0.8893GP + 0.448CP + 0.651CA 2

Where;

GP= 24 h of net gas production (mL 200 mg^{-1}).

CP= Crude protein (%).

EE= Ether extract (%).

CA= Ash content (%).

The methane content of the gas produced after 24-h incubation was determined using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel *et al*., 2008), using the following equation:

Methane production (mL)=

Total gas production (mL) × percentage methane (%) 3

Statistical analyses of the data obtained in the study were performed using the one-way analysis of variance method (ANOVA) in the SPSS 9.0 (2003) programme. To determine the significance of differences between means, Duncan's comparison test was used.

RESULTS AND DISCUSSION

The values obtained from the chemical analyses of the Phacelia plant harvested in different growth stages are shown in Table 1.

When the effects of harvest timing on the chemical components of Phacelia were evaluated (Table 1), it was found that the effects on all constituents except CT (condensed tannin) were highly significant ($p<0.001$). As the vegetative process of Phacelia progressed, the amount of dry matter increased due to development and maturation. The reason for the stageic increase in dry matter was the maturation of the plant and the associated increase in the amounts of cellulosic materials (ADF and NDF) in the cell wall. As the harvest stage progressed, an increase in hay

Table 1: Nutrient contents of phacelia in different growing stages (DM%).

Nutrient (%)	Harvesting stage				
	Pre flowering (I)	Early flowering (II)	Mid-flowering (III)	End flowering (IV)	*p*
DM	32.62±0.44[a]	37.77±1.02[b]	43.17±0.31[c]	54.58±2.59[d]	***
CA	11.18±0.25[c]	10.61±0.23[c]	9.60±0.28[b]	8.66±0.25[a]	***
CP	19.79±0.39[d]	18.86±0.06[c]	15.71±0.24[b]	14.76±0.11[a]	***
CF	2.80±0.05[c]	2.04±0.06[b]	1.94±0.08[b]	1.42±0.03[a]	***
NDF	38.97±0.23[a]	40.45±0.11[b]	41.67±0.24[c]	45.03±0.59[d]	***
ADF	28.58±0.22[a]	29.43±0.30[b]	31.09±0.19[c]	32.27±0.30[d]	***
CT	0.92±0.04	0.85±0.02	0.78±0.02	1.03±0.15	ns

DM: dry matter (%), CA: Crude ash (%), CP: Crude protein (%), CF: Crude fat (%), ADF: Acid detergent fiber (%), NDF: Neutral detergent fiber (%), CT: Condensed tannins (%),[abc] Difference between groups in the same column is statistically significant, p: Level of significance, ***$p<0.001$, ns: Not significant.

yield was observed. While the percentage of dry matter in the first stage was 32.62%, in the last stage it was 54.58%. The dry matter content of the samples were 32.62, 37.77 43.17 and 54.58% for the first, second, third and fourth stages, respectively and the difference between the stagestages was statistically significant (p<0.001). An increase in dry matter content was observed with the delay in harvesting stage and the differences between stages were highly significant. This result is consistent with the results of previous studies (Soya *et al*., 1999; Kamalak *et al*., 2014; Ayaşan *et al*., 2020a; Ayaşan *et al*., 2020b).

When examining the influence of harvest stage on CA %, the results for the first and second stages were statistically similar and there was no significant difference. The differences between the third and fourth stages were found to be highly significant (p<0.001). While the ash content in the first stage was 11.18%, it was 10.61% in the second stage. The CA rates in the third and fourth stage were 9.60 and 8.66%, respectively. The CA contents of Phacelia in all stages were higher than those reported in a previous study for alfalfa, vetch, pea, cloverleaf and dried rapeseed grasses (Canbolat and Kara, 2013). In their study with two different Phacelia varieties, researchers (Geren *et al*. 2007) generally reported similar average CA ratios as in this study. In other studies of quinoa and wild sainfoin in plants (Kaplan *et al*., 2017, Kamalak *et al*., 2014), the finding that CA content decreases as the plant matures was comparable to the data obtained in this study.

In this study, it was found that the CP amounts (DM%) of the different stages of the Phacelia plant varied from 14.76 to 19.79%. The crude protein content for the first, second, third and fourth stages were 19.79, 18.86, 15.71 and 14.76%, respectively. The highest crude protein content was 19.79% in the first stage and the lowest CP was 14.76% in the fourth stage. The difference between all stages in terms of CP ratios was significant (p<0.001). The results of chemical analysis of Phacelia showed that CP content decreased with the growth and maturity of the plant, which is consistent with the results of some previous studies (Soya *et al*., 1999; Kamalak *et al*., 2014; Kaplan *et al*., 2017; Ayaşan *et al*., 2021). In another study (Geren *et al*., 2007) on two different Phacelia cultivars, it was observed that the mean values of the ratio CP were lower than the values obtained for all four stages in this study. Similar to this study, other researchers Kamalak *et al*. (2014) and Ayaþan *et al*. (2021) also reported that there was a negative correlation between an increase in dry matter and a decrease in crude protein content.

The CF amounts (DM%) in the four different growing stages of Phacelia plant were studied, the crude fat content was 2.80, 2.04, 1.94 and 1.42%, respectively and the difference in these CF ratios between all stages was significant (p<0.001). Canbolat and Kara (2013) reported higher crude fat contents in alfalfa, vetch, pea, clover and canola than found in the study for Phacelia. In their study with wild clover grass, Kamalak *et al*. (2014) found that the ratio CF decreased with maturity. These results were similar to those of the present study. While the stageic increases in the rates CA, CP and CF were similar in our study and the ratios of these chemical compounds were high in the fistage, the ratios of these constituents decreased as the Phacelia plant developed and matured.

Table 1 show the NDF and ADF values for the different growing seasons of Phacelia. The NDF values were 38.97, 40.45, 41.67 and 45.03% for the first to ftages, respectively. The ADF values were 28.58, 29.43, 31.09 and 2.27%, respectively, in the succession. The increase in ADF and NDF values was similar to the increase in dry matter content. The ADF and NDF contents are among the factors that directly affect the quality of the forage. The increase in ADF and NDF contents, which are cell wall components, as a function of advancing harvest stage proved to be highly significant (p<0.001). Similar results were obtained by other researchers. It was found that as harvest time progressed, poorly digestible materials such as ADF and NDF increased and corresponding lignification occurred (Cassida *et al*., 2000; Kamalak *et al*., 2014; Kaplan *et al*., 2017, Ayaşan *et. al*. 2020a; Ayaşan *et al*., 2021). Lower ADF ratios than in our study were observed in alfalfa, vetch, pea, bird's-foot trefoil and colza dried grasses, but the NDF ratios observed in alfalfa, vetch and pea in the same study were similar to those in our study (Canbolat and Kara, 2013).

Gas production, methane production, metabolic energy and degree of organic matter digestion of Phacelia in different time stages are shown in Table 2. As shown in the table, the differences between the data of different stages were highly significant (p<0.001).

The highest value of daily *in vitro* gas production (GP) of Phacelia was recorded at the beginning of flowering, in the second stage, with 45.68 ml. The second highest value was obtained in the samples taken in the middle of the

Table 2: Gas production, methane production, metabolic energy and organic matterdigestibility of phacelia in different stages.

Stages	GP (mL)	CH_4 (mL)	CH_4 (%)	ME (MJ /kg DM)	OMD (%)
Pre flowering (I)	30.56±0.33a	0.93±0.02a	3.05±0.08a	7.49±0.05a	51.68±0.32a
Early flowering (II)	45.68±0.38c	5.33±0.06c	11.66±0.10b	9.54±0.05d	64.95±0.37d
Mid flowering (III)	42.08±0.71b	4.74±0.07b	11.28±0.16b	8.90±0.11c	60.51±0.74c
End flowering (IV)	41.84±0.73b	5.38±0.11c	12.85±0.21c	8.61±0.08b	58.48±0.54b
p	***	***	***	***	***

GP: Gas production in 24 hours (mL), CH_4: Methane production (mL/%), ME: Metabolic energy, OMDD: Organic matter digestibility (%), abc: Difference between groups in the same column is statistically significant. p: Level of significance, ***p<0.001.

flowering stage (42.08 ml), corresponding to the third stage. The difference in daily *in vitro* gas production between the studied stages proved to be highly significant ($p<0.001$). The daily gas production of Phacelia ranged from 30.56 mL to 45.68 mL in all four stages. A previous study by Canbolat and Kara (2013) found higher daily gas production levels in alfalfa, vetch, pea, bird's-foot trefoil and colza dried grasses in comparison to the levels found in our study. Other studies on quinoa and wild trefoil (Kaplan *et al*. 2017) and Kamalak *et al*. (2014) showed that *in vitro* gas production decreased with increasing maturity. In our study, these values increased in a general trend. The daily gas production values of Phacelia plant for the preflowering stage in this study were very low compared to the values reported for quinoa and wild trefoil (Kaplan *et al*., 2017; Kamalak *et al*., 2014 Ayaþan *et al*. 2020b; Ayaşan *et al*., 2021).

In this study, CH_4 concentrations for the first, second, third and fourth stages were 0.93, 5.33, 4.74 and 5.38 mL, respectively, while CH_4 ratios were 3.05, 11.66, 11.28 and 12.85%, respectively. The highest CH_4 concentration (5.38 mL) and CH_4 ratio (12.85%) were obtained in the samples from the second stage. The lowest CH_4 concentration (0.93 mL) and CH_4 ratio (3.05%) were found in the samples from the first stage. In a study on quinoa and wild trefoil (Kaplan *et al*. 2017; Kamalak *et al*. 2014), the amount (mL) and percentage (%) of CH_4 were found to decrease with maturity. However, in previous study, these values generally increased. It was found that the amount (mL) and percentage (%) of CH_4 were higher in quinoa and wild trefoil Kaplan *et al*. (2017), Kamalak *et al*. (2014) compared to Phacelia. In addition to, Gautam *et al*. (2018) reported that the addition of 10% deoiled ajwain (*Trachyspermum ammi*) meal (DOAM) to the concentrate in the mixture resulted in lower methane production. Osita *et al*. (2019) conducted a study to determine the effects of adding yeast (*Saccharomyces cerevisiae*) to his diet on methane production. Based on the results, it was suggested adding Saccharomyces Cerevisiae to the legume diet to improve methane emission. Jafari *et al*. (2020) investigated the effect of bamboo leaf (BL) on rumen methane gas production *in vitro*. They reported that methane gas production (mL/250 mg DM) decreased at a decreasing rate with higher BL levels. Moreover, Murillo-Ortiz *et al* (2018) reported that net gas production decreased linearly when alfalfa hay was substituted, while methane and CO_2 production decreased linearly with the addition of water hyncinth. The results showed that WH has emerged as a promising alternative to reduce methane production in ruminants. In addtion, Kaur *et al.* (2017) *in vitro* analysis revealed that the net gas production was the lowest in P. minor seeds (216.37 L/kg DM/24 h). Methane production (L/kg DM/24 h) from P. minor seeds (43.03) were lower than wheat (54.33) and barley (57.35). Dey *et al*. (2022) investigated the *in vitro* fermentation model of the stovets obtained from three different new sorghum (*Sorghum bicolor* L.) cultivars in buffalo. The fermentation pattern of brown midrib sorghum stovers reported higher total gas production than normal and sweet sorghum stovers. Apart from all these research studies mentioned above, Sarkar *et al* (2018) showed significant increase in total gas (mL/g DM) between different diets, CH_4 (%, mL/24h and mL/100 mg DDM) and NH_3-N (mg/dL) on supplementation of Aegle leaves if any of the diets. The values of CH4 (%, mL/24h and mL/100 mg DDM) and NH_3-N (mg/dL) on supplementation of Aegle leaves if any of the diets were found to be not significant.

The ME (metabolic energy) amounts (DM%) of Phacelia plant for the first, second, third and fourth stages were 7.49, 9.54, 8.90 and 8.61 MJ/kg DM, respectively. On the other hand, in a previous study (Canbolat and Kara 2013) higher metabolic energy contents were found in alfalfa, vetch, pea, bird's-foot trefoil and colza dried grasses. Another study by Kamalak *et al*. (2014), conducted on wild trefoil, found that the amount of metabolic energy decreased with increasing maturity. However, in our study, this parameter varied with advancing maturity with different tendency.

In study, the OMD (%) of Phacelia plant for the first, second, third and fourth stages were 51.68, 64.95, 60.51 and 58.48%, respectively. The lowest value was found in the pre-flowering stage, while the highest value was obtained at early flowering stage. In a previous study, it was found that the organic matter digestibility of alfalfa, vetch, pea, bird's-foot trefoil and colza dried rapeseed grass were higher than the values obtained in our study with Phacelia. In addition, other studies on quinoa and wild trefoil (Kaplan *et al*. 2017; Kamalak *et al*. 2014) found that ripening reduced organic matter digestibility. In contrast, this study found that ripening generally increased digestion.

CONCLUSION

According to the results of this study, it is appropriate to harvest the Phacelia plant, which is known as an important source of nectar in the world and Turkey, in the pre-flowering stage in terms of GP, crude ash, crude protein, crude fat, CH_4 amount and CH_4 ratio. Due to the high amounts of ME and OMD, it is appropriate to harvest in the second stage, *i.e.*, at early flowering. Nevertheless, it is important to investigate the effects of harvest stage of Phacelia on feed value and consumption of the crop by animals with new *in vitro* and *in vivo* studies.

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REFERENCES

AOAC, (1990). Association of Official Analytical Chemists. Official Method of Analysis. 15th. Ed. Washington. DC, USA., pp. 66-88.

Ayaşan, T., Çetinkaya, N., Aykanat, S. and Çelik, C. (2020a). Nutrient contents and *in vitro* digestibility of different parts of corn plant. South African Journal of Animal Science. 50(2): 302-309.

Ayaşan, T., Sucu, E., Ülger, İ., Hızlı, H., Cubukcu, P. and Ozcan, B.D. (2020b). Determination of *in vitro* rumen digestibility and potential feed value of tiger nut varieties. South African Journal of Animal Science. 50(5): 738-744.

Ayaşan, T., Ulger, İ., Cil, A.N., Tufarelli, V., Laudadio, V. and Palangi, V. (2021). Estimation of chemical composition, *in vitro* gas production, metabolizable energy, net energy lactation values of different peanut varieties and line by Hohenheim *in vitro* gas production technique. Semina: Ciencias Agrarias 42(2), 907-920.

Canbolat, Ö. and Kara, H. (2013). Comparison of *in vitro* gas production, metabolizable energy, organic matter digestibility and microbial protein production of some legume hays. Uludag Journal of the Faculty of Agriculture of the University 27(2): 71-81.

Cassida, K.A., Griffin, T.S., Rodriguez, J.S., Patching, C., Hesterman, O.B. and Rust, S.R. (2000). Protein degradability and forage quality in maturing alfalfa, red clover and birds foot trefoil. Crop Science January. 40: 209-215.

Dey, A., Paul, S.S., Umakanth, A.V., Bhat, B.V., Lailer, P.C. and Dahiya, S.S. (2022). Exploring feeding potential of stovers from novel sorghum (*Sorghum bicolor* L.) cultivars by *in vitro* fermentation pattern, gas production, microbial abundance and ruminal enzyme production in buffalo. Indian Journal of Animal Research. 56(1): 51-57. DOI: 10.18805/IJAR.B-4193.

Gautam, M., Sehgal, J.P., Mohini, M., Gupta, R., Varun, T.K. and Sarkar, S. (2018). *In vitro* nutritional evaluation and methane production of deoiled ajwain meal as a potential ruminant feed ingredient. Indian Journal of Animal Research. 52(4): 569-573. doi: 10.18805/ijar.v0i0f.3788.

Geren, H. and Kaymakkavak D. (2007). Effects of different row spacings on the herbage yield and some other yield and quality characteristics of phacelia (*Phacelia tanacetifolia* Bentham.) varieties. Journal of Ege University Faculty of Agriculture. 44(1): 71-85.

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

Jafari, S., Goh, Y.M., Rajion, M.A. and Ebrahimi, M. (2020). Effects of polyphenol rich bamboo leaf on rumen fermentation characteristics and methane gas production in an *in vitro* condition. Indian Journal of Animal Research. 54(3): 322-326. DOI: 10.18805/ijar.B-771.

Johnson, D.E., Hill, T.M., Carmean, B.R. and Lodman, L.W. (1991). New Perspective on Ruminant Methane Production. In: Energy Metabolism of Farm Animal. [Wenkand, C., Boessinger, M. (ed)], Zurich, Switzerland.

Kamalak, A., Kaplan, M., Özkan, C.O. and Atalay, A.İ. (2014). Effect of vegetative stages on potential nutritive value, methane production and condensed tannin content of onobrychis caput-gallu hay. Journal of Harran University Veterinary Faculty. 3(1): 1-5.

Kamra, D.N., Agarwal N. and Chaudhary, L.C. (2006). Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds. International Congress Series. 1293: 156-163.

Kaplan, M., Üke, Ö., Kale, H. and Kamalak, A. (2017). Olgunlaþma döneminin kinoa (*Chenopodium quinoa* willd.)'da o verimi ve kalitesi ile gaz ve metan üretimine etkisi. KSU Journal of Natural Sciences. 20(1): 42-46.

Kaur, J., Thakur, S.S. and Singh, M. (2017). Nutritional value of Phalaris minor seeds and its comparison with conventional cereal grains for livestock feeding. Indian J. Anim. Res. 51(5): 887-891. DOI: 10.18805/ijar.v0iOF.8453.

Makkar, H.P.S., Becker, K., Abel, H.J. and Szegletti, C. (1995). Degradation of condensed tannins by rumen microbes exposed to quebracho tannins (QT) and effects of QT the Rusitec. Journal Science and Food Agriculture. 69: 495-500.

Makkar, H.P.S., Blümmel, M. and Becker, K. (1995). Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins and their implication in gas production and true digestibility in *in vitro* techniques. British Journal of Nutrition. 73(6): 897-913.

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

Murillo-Ortiz, M., Herrera-Torres, E., Corral-Luna, A. and Pamanes-Carrasco, G. (2018). Effect of inclusion of graded level of water hyacinth on *in vitro* gas production kinetics and chemical composition of alfalfa hay based beef cattle diets. Indian Journal of Animal Research. 52(9): 1298-1303. DOI: 10.18805/ijar.11417.

Osita, C.O., Ani, A.O., Ezema, C., Oyeagu, C.E., Uzochukwu, I.E. and Ezemagu, I. E. 2019. Growth, lipid profile and methane production by sheep fed diets containing yeast (*Saccharomyces cerevisiae*). Indian Journal of Animal Research. 53(11): 1485-1488. DOI: 10.18805/ijar.B-969.

Saðlam, N.E., Düzgüneþ, E. and Balık, İ. (2008). Global warming and climatic changes. Ege University Journal of Fisheries and Aquatic Sciences. 25(1): 89-94.

Saglamtimur, T., Tansý, V. and Baytekin, H.A. (1989). Study on the effect of harvesting time on plant height and grass yield in beech (*Phacelia tanacetifolia benth*.) grown as a winter intermediate crop in Cukurova conditions. Cukurova University Journal of Agriculture Faculty. 4(1): 76-83.

Sarkar, S., Mohini, M., Mondal, G., Pandita, S., Nampoothiri, V.M. and Gautam, M. (2018). Effect of supplementing Aegle marmelos leaves on *in vitro* rumen fermentation and methanogenesis of diets varying in roughage to concentrate ratio. Indian Journal of Animal Research. 52(8): 1180-1184. DOI: 10.18805/ijar.B-3331.

Soya, H., Doğrucu, F., Geren, H. and Kır, B. (1999). A Study on the Effects of Different Cutting Times on the Yield and Yield Characteristics of Common Vetch (*Vicia sativa*) and Hairy Vetch (*Vicia villosa*). Turkey 3rd Field Crops Congress, 15-18 November 1999, Adana, Volume III, Meadow Pasture Forage Crops and Edible Grain Legumes. 92-95.

SPSS (1998). SPSS for Windows Version 9.0; Chicago:İllinois , USA.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M.D.E. and Haan, C. (2006). Livestock's Long Shadow: Environmental Issues and Options. FAO, Food Agriculture Organization of the United Nations.

Van Soest, P.J., Robertson, J. and Lewis, B. (1991). Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74: 3583-3597.

Van Soest, P.J. (1994). Nutritional Ecology of the Ruminant (2nd Ed.). p. 528. Cornell University Press. Ithaca.

Wallace, R.J. (2004). Antimicrobial properties of plant secondary metabolites. Proceedings of the Nutrition Society. 63: 621-629.