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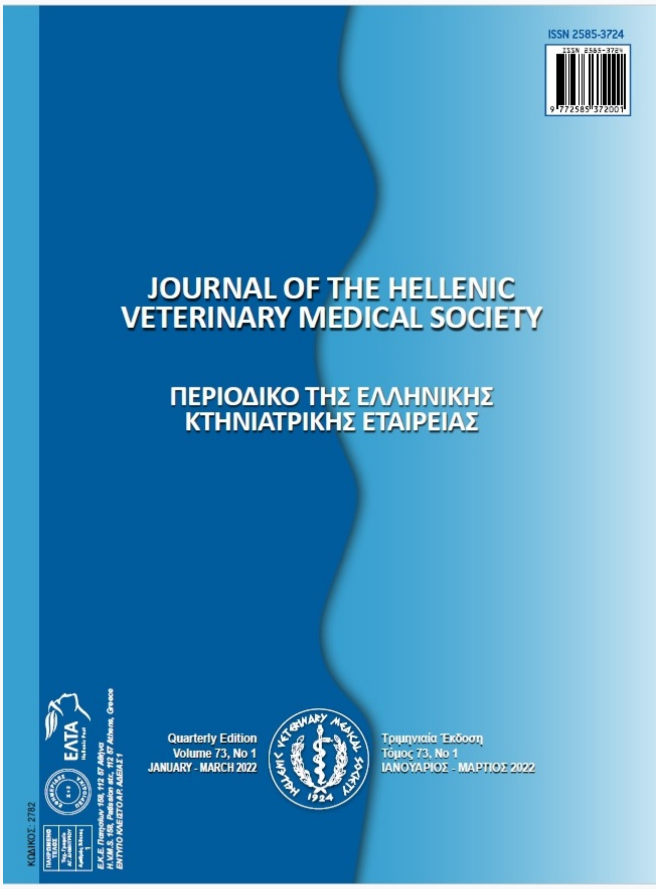

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Research article
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Determination of Nutritional Value of Alfalfa Varieties and Lines by Using the In Vitro Method and Gas Production Technique

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ABSTRACT: This study was organized to determine the nutritional value of different alfalfa varieties and lines selected within the scope of the "Cukurova Region Alfalfa Breeding Project", conducted at the Eastern Mediterranean Agricultural Research Institute, by using the in vitro gas production technique. In the project, Nimet was certified as a new variety. The used alfalfa lines were YSH 26-12, YSH 23-9, YSH 21-1, YSH 27-9, YSH 37-12, YSH 35-11, YSH 28-6, YSH 16-11, YSH 14-3, and YSH 11-4. The certified Nimet variety was used as a control. Alfalfas have been harvested in April, at the beginning of blooming. Chemical composition, metabolic energy (ME), net energy lactation (NEL), and organic matter digestibility (OMD) of the certified Nimet variety with 10 different alfalfa lines used in the experiment were determined by Hohenheim in vitro gas production technique. The incubation times in the Hohenheim gas production technique are 3, 6, 9, 12, 24, 48, 72 and 96th hours. Crude protein (CP), crude cellulose (CC), NDF, and ADF contents of the alfalfa varieties and lines ranged between 19.06-22.40%, 24.90-33.30%, 33.16-45.73%, and 30.77-39.75%, respectively. After 96-hour incubation, the highest total gas production (GP) was found at the YSH 11-4 line (45.32 ml) ($P < 0.05$). While ME, OMD and NEL contents were found to be high at the YSH 11-4 line, ME and OMD values were statistically different from the YSH 28-6, YSH 16-11, YSH 14-3, and YSH 21-1 lines. On the other hand, NEL values were determined different from YSH 23-9, YSH 21-1, YSH 28-6, YSH 16-11, YSH 14-3 lines.

Keywords: Alfalfa, variety, Line, Nutrient chemical composition, In vitro gas production, Digestibility

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INTRODUCTION

One of the most important problems to solve in our country's animal husbandry is the difficulties in finding quality and cheap roughage that the animals need. In order to deal with these problems, it is necessary to use meadows and pastures properly, to reclaim them, and to meet the needs of animals by increasing the production areas of forage crops (Ünalp, 2015).

One of the good-quality roughage sources is alfalfa too. Alfalfa is an important forage crop both in terms of CP and in terms of yield. The harvest of alfalfa 6-7 times a year in Adana conditions and the changing of the nutrient material depending on the harvest time are the issues to be taken into account for those who prepare rations in animal feeding. In addition, the fact that the alfalfa is dry and its fresh grass is higly palatable is a preference reason for animals (Alvaro et al., 2019).

In the Eastern Mediterranean Agricultural Research Institute, studies are being carried out within the scope of the "Cukurova Region Alfalfa Breeding Project". Clonal reproduction of promising alfalfa genotypes is needed at different stages of alfalfa breeding studies. Within the scope of the Alfalfa Breeding Research Project, the appropriate method for clonal reproduction of 16 different alfalfa genotypes selected for the aim of variety development was investigated (Avcı et al., 2017).

The Nimet variety, which was developed at the Research Institute and registered, is a variety that is grown successfully in Mediterranean and Southeastern Anatolia regions, contains 18-20% CP, 32-36% ADF, 40-44% NDF, and whose seed yield is 2-2.5 tons/da (Anonim, 2019).

The YSH 26-12, YSH 23-9, YSH 21-1, YSH 27-9, YSH 37-12, YSH 35-11, YSH 28-6, YSH 16-11, YSH 14-3, YSH 11-4, which were used in the study, are alfalfa lines under development at the Institute.

In this study, using in vitro gas production technique in Adana conditions, it was aimed to identify the nutritional value of different alfalfa varieties and lines (Nimet, YSH 26-12, YSH 23-9, YSH 21-1, YSH 27-9, YSH 37-12, YSH 35-11, YSH 28-6, YSH 16-11, YSH 14-3, YSH 11-4) selected within the context of the "Cukurova Region Alfalfa Breeding Project" carried out in the Eastern Mediterranean Agricultural Research Institute.

MATERIALS AND METHODS

Animal Material

In the present study, rumen liquids and contents obtained from the slaughterhouse were brought in thermoses to the Zootechnical Laboratory of Cukurova University Faculty of Agriculture and used in the *in vitro* gas production system.

Forage Material

Different alfalfa varieties and lines found at the Eastern Mediterranean Agricultural Research Institute was used as forage material. The used forages were analyzed in a way that they were three-repetition.

In Vitro Gas Production Technique and Forage Analyses

100:1 ml (Fortuna, Germany) gas syringes were used in the application of the *in vitro* gas production technique. While the analyses of DM, CP, CF, CC and CA in forage materials used in the experiment were conducted as reported by AOAC (2001), analyses of the ADF and the NDF were conducted as reported by Van Soest and Wine (1967). On the other hand, the values of nitrogen-free extract (NFE) were determined by calculation. All chemical analyses were performed as three-repetition.

In vitro gas production technique was applied in determining the total gas quantities of forages (Menke et al., 1979; Ørskov and McDonald, 1979; Blümmel and Ørskov, 1993). In the experiment, first of all, nutrient material analyses were made in alfalfa varieties and lines in a way that it was 3-repetition. Then, OMD was determined by the in vitro gas production technique. OMD, ME and NEL values of alfalfa varieties and lines were calculated based on 24-hour gas production values, and gas production parameters and pH values were also determined as a result of 96-hour incubation.

Application of the In vitro Gas Production Technique

The alfalfa samples were grounded in a way that they can pass through a 1 mm sieve and the dry forage matter (200 mg DM) was weighed in approximately 250 mg air and placed at the bottom of the injector. Just before the rumen liquid was taken, the medium prepared by mixing 0.1 mL micro-mineral solution, 200 mL rumen buffer solution, 200 mL macro-mineral solution, 1.0 mL resazurin solution and 40 mL reduction solution into 400 mL pure water was kept in a water bath at 39 ºC under CO_2. The rumen liquid taken from the slaughterhouse was transported to the laboratory in thermoses in a short span of time and the rumen liquids were filtered through two layers of cheesecloth into a 2-liter erlenmeyer flask, which was heated at 39 °C and fed by carbon dioxide. One-part rumen liquid was mixed with two parts medium, and carbon dioxide gas was constantly given into the mixture. 30 mL from the rumen liquid and medium mixture was added to each injector. Readings were performed at the 3, 6, 9, 12, 24, 48, 72, and 96th hours. In addition, after 96 hours of incubation, determination of pH was carried out in the liquid remaining in the injectors.

Gas production parameters were calculated according to the following model with the help of the PC package program called NEWAY.

$$GP=a+b\ (1-e^{-ct})$$

where;

a: amount of gas (ml) consisting of the immediately soluble fraction,

b: the amount of gas formed based on time (ml),

a: gas production speed,

a+b: potential gas production (ml),

t: incubation period (hours),

GP: gas production in “t” time

Organic matter digestibility (OMD, %) was calculated by using gas production quantity (GP) at the 24th hour, CP (g / kg DM), and CA (g / kg DM) values according to the following formula (Menke et al., 1979).

OMD, % = 14.88 + 0.889 GP + 0.45 CP + 0.065 CA

In order to determine the NEL (MJ / kg DM) (Menke and Steingass, 1988) and ME (MJ/kg DM) (Close and Menke, 1986) contents of the forages, the following equation was used.

NEL= 0.075 GP + 0.087 CP + 0.161 CF + 0.056 NFE - 2.422

ME= 1.06 + 0.157 GP + 0.00884 CP + 0.022 CF - 0.0081 CA

where;

GP: Gas production after 24-hour incubation (ml / 200 mg DM), CP: g / kg DM, CF: g / kg DM, CA: g / kg DM, NFE: g / kg DM

Statistical Analysis

To determine the differences between the means for the aim of statistical evaluation of the data obtained from the research, the analysis of variance (General Linear Model) was performed using the SPSS (1999) package program. The Duncan’s multiple comparison test was also used to determine the significance levels of the differences.

RESULTS

While nutrient analysis of alfalfa varieties and lines are given in Tables 1 and 2, their average gas productions at different times are presented in Table 3. On the other hand, whereas GP, ME and OMD of the alfalfa varieties and lines are given in Table 4, their effect on gas production is shown in Figure.

Table 1. Crude ash, crude protein of alfalfa varieties and lines

Varieties and lines	CA, DM %	CP, DM %
Nimet Variety	10.055	21.935
YSH 26-12 line	10.185	22.395
YSH 23-9 line	9.54	21.40
YSH 21-1 line	9.14	19.06
YSH 27-9 line	9.36	20.09
YSH 37-12 line	9.00	20.00
YSH 35-11 line	9.96	20.00
YSH 28-6 line	9.82	20.33
YSH 16-11 line	9.59	19.75
YSH 14-3 line	8.90	18.89
11-4 line	10.26	22.26

CA: Crude ash; CP: crude protein; DM: Dry matter

Table 2. ADF, NDF, Crude cellulose and hemicellulose contents of alfalfa varieties and lines

Varieties and lines	ADF, DM %	NDF, DM %	CC, DM %	HES, DM %
Nimet Variety	31.92	37.32	24.90	5.40
YSH 26-12 line	34.21	37.09	26.82	2.88
YSH 23-9 line	32.85	36.92	27.02	4.07
YSH 21-1 line	39.75	45.73	33.31	5.98
YSH 27-9 line	36.27	40.30	29.82	4.03
YSH 37-12 line	39.00	43.70	31.91	4.70
YSH 35-11 line	32.30	37.30	26.08	5.00
YSH 28-6 line	35.95	38.08	28.52	2.13
YSH 16-11 line	38.51	42.15	31.25	3.64
YSH 14-3 line	38.84	42.63	32.50	3.79
11-4 line	30.77	33.16	23.90	2.39

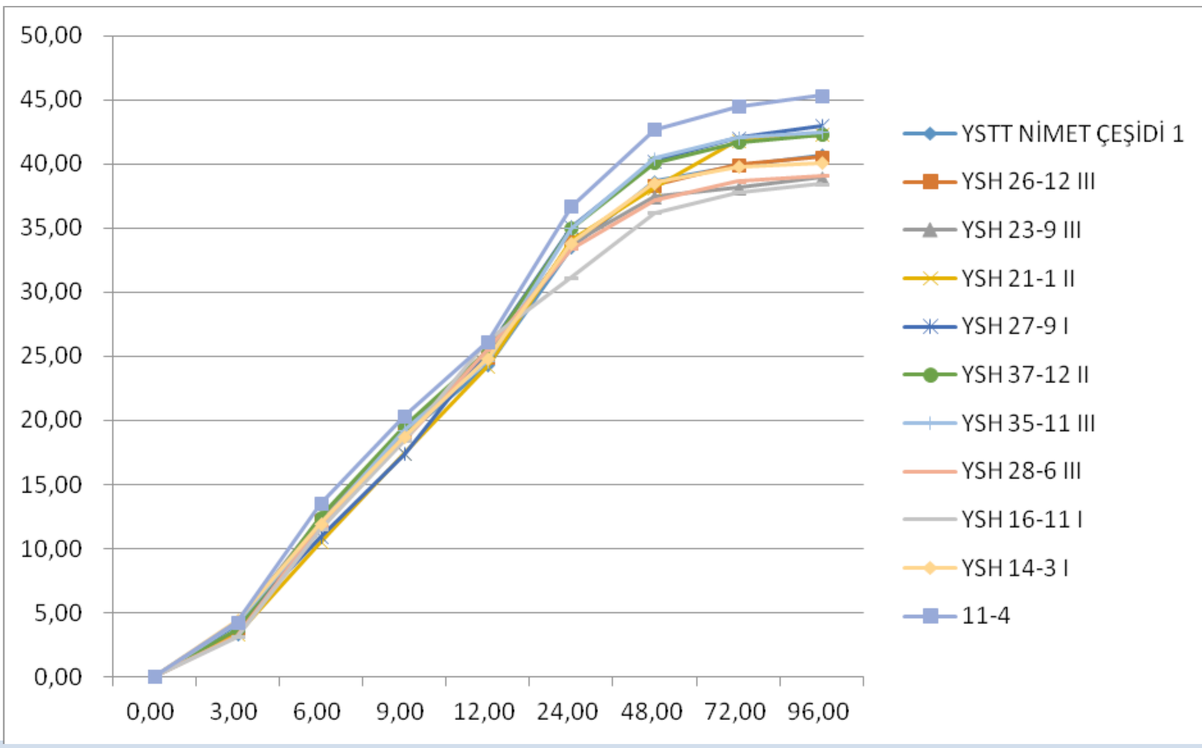

Figure 1. Effect of alfalfa varieties and lines on gas production (ml)

Table 3. Average gas production of alfalfa varieties and lines at different times

Varieties and lines	0.00	3.00	6.00	9.00	12.00	24.00	48.00	72.00	96.00
Nimet Variety	0.00	3.37	11.86	18.87	24.31	33.52	38.64^{bc}	39.90^{bc}	40.71^{bc}
YSH 26-12 line	0.00	3.63	12.25	19.07	24.90	33.88	38.34^{bc}	39.92^{bc}	40.54^{bc}
YSH 23-9 line	0.00	4.22	12.43	19.18	25.26	33.82	37.46^{bc}	38.17^{bc}	38.97^{bc}
YSH 21-1 line	0.00	3.34	10.50	17.45	24.26	34.09	38.13^{bc}	41.84^{abc}	42.28^{abc}
YSH 27-9 line	0.00	4.09	10.93	17.40	25.55	35.11	40.23^{ab}	42.02^{ab}	43.01^{ab}
YSH 37-12 line	0.00	3.70	12.47	19.60	25.52	35.00	40.08^{abc}	41.68^{abc}	42.30^{abc}
YSH 35-11 line	0.00	4.23	11.94	19.25	24.63	35.01	40.44^{ab}	42.04^{ab}	42.49^{abc}
YSH 28-6 line	0.00	3.16	11.74	18.52	25.53	33.40	37.22^{bc}	38.64^{bc}	39.08^{bc}
YSH 16-11 line	0.00	3.14	11.53	18.45	26.32	31.16	36.21^{c}	37.81^{c}	38.43^{c}
YSH 14-3 line	0.00	4.42	11.95	18.74	24.85	33.83	38.56^{bc}	39.81^{bc}	40.08^{bc}
11-4 line	0.00	4.28	13.52	20.37	26.18	36.67	42.71^{a}	44.51^{a}	45.32^{a}
SEM	0.00	0.38	0.85	0.88	0.92	1.04	1.18	1.22	1.25
Significance level	0.00	0.17	0.56	0.50	0.83	0.13	0.04	0.02	0.02

Table 4. Gas production, metabolic energy and organic matter digestibility of alfalfa varieties and lines

Varieties and lines	pH	a	b	c	RSD	OMD	ME, MJ / kg DM	NEL, MJ / kg DM
Nimet Variety	6.850	-7.69^{abc}	47.597	0.09^{abcd}	.693	56.32^{abc}	8.15^{ab}	4.90^{ab*}
YSH 26-12 line	6.833	-7.86^{abc}	47.610	0.09^{abcd}	.773	56.65^{abc}	8.20 ^{ab}	4.86^{ab}
YSH 23-9 line	6.810	-8.07^{abc}	46.493	0.1^{abc}	.820	56.32^{abc}	8.16^{ab}	4.75^{b}
YSH 21-1 line	6.843	-7.42^{ab}	48.667	0.08^{d}	1.490	55.23^{bcd}	8.04^{bc}	4.70^{bc}
YSH 27-9 line	6.833	-7.47^{ab}	49.673	0.08^{cd}	1.433	56.71^{abc}	8.24^{ab}	4.9^{ab}
YSH 37-12 line	6.830	-7.833	49.367	0.09^{abcd}	.790	56.43^{abc}	8.20^{ab}	4.89^{ab}
YSH 35-11 line	6.845	-6.61^{a}	48.607	0.087$_{bcd}$	.593	58.26^{ab}	8.43^{ab}	4.98^{ab}
YSH 28-6 line	6.818	-9.63^{bc}	48.047	0.10^{ab}	1.190	55.26^{bcd}	8.02^{bc}	4.78^{b}
YSH 16-11 line	6.827	-9.91^{d}	47.177	0.11^{a}	1.780	52.95^{d}	7.67^{c}	4.41^{c}
YSH 14-3 line	6.850	-6.84^{a}	46.547	0.09^{abcd}	.717	54.88^{cd}	7.99^{bc}	4.73^{b}
11-4 line	6.848	-6.62^{a}	51.123	0.08^{cd}	.763	59.32^{a}	8.60^{a}	5.14^{a}
SEM	.012	.732	1.311	.006		.925	.142	.106
Sig	.361	.058	.370	.057		.009	.017	.013

*a, b: $P<0.05$

DISCUSSION

In our study, the CP values of the alfalfa varieties and lines were between 18.89% (YSH-14-3) and 22.40% (YSH-23-9). It has been reported that the Nimet variety contains 90.41 % DM, 19.83% CP, 1.64% CF, 23.47% CC, 48.39% NDF, 37.06% ADF, 11.29% ADL (Acid detergent lignin), 60.04% DDM, and 115.4% relative feed value in its structure (Central Directorate of Seed Registration and Certification, 2016).

Engin and Mut (2017), who investigated the nutrient content of the Nimet variety grown under Yozgat conditions, stated that the Nimet variety contained 23.3-25.1% CP, 41.3-42.2% NDF, and 28.8-29.7% ADF in its structure. Whereas the average CP (21.935%) obtained in our study was consistent with the CP (20.62-23.76%) determined by Töngel and Ayan (2010), it was lower than the CP rate identified as 22.67% by Saruhan and Kuşvuran (2011), and it was higher than the CP rates determined by Lemes et al., (2016) 17.37%; Kır and Soya (2008) (17.86-20.26%), Avcı et al., (2009) (17-18%), and Yılmaz (2011) (17.53%). Gökalp et al., (2017) also stated that the average CP rates of alfalfa varieties varied between 17.51% and 18.00%. The differences between the CP values found in the literature and the CP values found in this study might have been due to the different ecological conditions of the place where the study was conducted, the delay of harvest time, and the differences in total precipitation and temperature during the vegetation.

In their study, in which they described 16 pieces of alfalfa hay produced in Kırıkkale region as good and bad quality, Güngör et al., (2008) found DM, CP, CF, CC, CA, ADF and ADL values of the good-quality alfalfas as 92.87, 20.26, 2.33, 24.71, 8.74, 33.52, and 8.26%, respectively, while they found the same values for the bad-quality alfalfas as 91.79, 12.11, 1.47, 30.62, 10.57, 39.64, and 9.92%, respectively.

In our study, alfalfa varieties and lines were not subjected to crude fat (CF) analysis since the instrument was out of order. While Şeker (2002), Abaş et al., (2005) and Kara and Deniz (2005) found the CF amounts in alfalfa hay as 1.48, 2.34, and 1.75%, respectively, Dale and Batal (2005) reported the same value as 1.7-3.5%. On the other hand, Ünalp (2015) stated that the CF in alfalfa differed according to different reaping times and vegetative periods. He found that the CF content of alfalfas at the beginning of blooming and during the full blooming and pod setting stages varied between 1.39 and 1.52%. Whereas the CA value for alfalfa varieties and lines was the lowest with 8.90% at the YSH 14-3 line, line 11-4 had the highest CA value with 10.26 %. In a research, it was stated that CA value of alfalfa was 8.40-9.52% (Kavut and Avcıoğlu, 2017).

The ADF is an indication of the digestibility of roughages and is determined in forages according to Van Soest (1963). ADF value (31.92%) of the variety in our study was consistent with the ADF values found in alfalfa by various researchers (Kamalak, 2005), (27.36%) ; Basbağ et al., (2009), (16.8%-33.3%)). However, it was also found that ADF value determined in this study was lower than the values found by some researchers (Kır and Soya, 2008), (35.16-36.03%)) and it was also higher than the values found by some other researchers (Canbolat et al., 2013) (26.60%)). Erbeyi (2017), who reported that the ADF values of different alfalfa varieties vary by year, found that the ADF rates ranged between 21.55%-25.87% and 26.81%-30.12%. In addition, it was expressed that the forage materials examined in terms of ADF were in compliance with the results of alfalfa dried fodders (28-37%) in the internationally accepted reference tables (Perry *et al.,* 2004). Stavarache et al., (2012) stated that the ADF rates in the whole plant differed by the time of fertilization and harvest, and they observed that the ADF rates ranged between 42.3% and 52.8%.

Although NDF contains cellulose, lignin and hemicellulose in its structure, digestion of it is difficult in terms of ruminant animals. Low NDF values in forages are a desired feature for animals and are associated with the increasing of the animal forage consumption. Engin and Mut (2017) reported that the NDF, ADF and HES contents of the Nimet variety received values of 41.3-42.2%, 28.8-29.7%, and 11.6-13.4%, respectively. Avcı et al., (2011) stated that the NDF values (43.6-48.7%) of alfalfa varieties and lines received an average value of 46.7% and found that the lines with the highest NDF values among the lines had a progressive morphological development. The same researchers stated that the average NDF contents of 2 types of alfalfa and 32 selected lines were 45.5% and 47.1%, respectively. Erbeyi (2017) found that NDF rates differed by years and this value ranged between 36.69-40.64% and 38.07-41.87%. The differences observed in terms of NDF among studies in the literature are due to ecological conditions, differences in terms of variety, and applied cultural practices.

In the study, CC values of alfalfa varieties and lines showed statistically significant differences and CC ranged between 24.90 and 33.31%. Özyiğit and Bilgen (2006) determined that CC value in alfalfa varied according to reaping times and it was 18.88% on average. Çerçi et al., (2004) found the CC value in alfalfa to be 23.13%. Ünalp (2015) reported that CC received values between 28.99% and 41.86% according to the harvest times.

The crude ash rates received values between 8.90 and 10.26% in our research. In a research on this subject, it was found that CA value in alfalfa varies according to the harvest, cutting times, but its average was 9.07% (Özyigit and Bilgen, 2006). Ünalp (2015) stated that in different cutting times and vegetation periods, CA content of alfalfa samples was statistically significant during full bloom and pod setting periods and that CA values were between 7.18 and 10.95%.

Hemicellulose values (HES) showed variance between 2.13% and 5.98% in alfalfa varieties and lines. Nikolova et al., (2018) found the HES value in alfalfa to be between 6.41% and 8.52%. The difference between the HES value we obtained in the experiment and the HES values of the researchers in the literature is due to the differences in the application of biological products to alfalfa.

The average gas production of alfalfa varieties and lines at different times is shown in Table 2. In Hohenheim gas production technique, incubation times are 3, 6, 9, 12, 24, 48, 72 and 96th hours. There was no difference between alfalfa varieties and lines in terms of gas production until the 48th hour. After the 48th hour, while the highest gas production was detected on the YSH 11-4 line, the lowest gas production was detected on the YSH 16-11 line with 36.21 ml / 200 mg DM. The 96-hour gas production of the forages ranged between 38.43 and 45.32 ml, and the difference between alfalfa varieties and lines was found to be statistically significant. 96-hour gas production received the highest value (45.32 ml) at the YSH-11-4 line, while it received the lowest value (38.43 ml) at the YSH-16-11 line. The reason that the gas production value of an alfalfa variety or line is low is that the NDF and ADF values of the alfalfa are high.

The pH values, a and b values of alfalfa varieties and lines did not differ statistically. c is the gas production rate of insoluble fractions; while time-dependent gas production of YSH-16-11 (c) was found the highest with 0.11, it was found the lowest (0.08) at YSH-21-1, 11-4, and YSH-27-9 alfalfa lines (Table 3). Aksoy and Yilmaz (2003), who investigated the OMD in some alfalfa varieties, stated that OMD values in the first cutting varied between 39.04-47.67% and OMD values in the second cutting varied between 48.15-54.21%. On the other hand, in our experiment, OMD ranged between 52.95 and 59.32%. In this difference, the variety used and the line types, the cutting time of the alfalfa, and the fact that regions from which alfalfa samples were taken were different played an active role. Çerçi et al., (2004) stated that alfalfa's dry and organic matter digestion values obtained at the end of 24-hour incubation by enzyme technique were 44.19% and 43.27%. Çerçi et al., (2004), who investigated the OMD of forages by nylon bag technique, expressed that OMD of alfalfa at the 48th hour was 61.72% and this value was 60.85% at the 24th hour.

The increase in the amount of gas produced by the 11-4 alfalfa line in the 24th hour together with CP content increased the OMD level (59.32%). The fact that alfalfa lines such as YSH-16-11 and YSH-21-1 were rich in nutrients such as NDF and ADF, which are difficult to dissolve in rumen, suppressed the microbial fermentation and reduced the amount of OMD.

Metabolic energy is a parameter obtained using criteria such as CP, CF, and CA. In our study, the ME value in alfalfa varieties and lines was found as 7.67-8.60 MJ / kg DM. The ME values that we obtained in the experiment were lower than the values of 13.73-19.54 MJ/kg DM determined by Aksoy and Yilmaz (2003) for alfalfa varieties. The harvest time of the alfalfa, analysis method, and regions were effective on this difference. Whereas the NEL value was found highest on the 11-4 alfalfa line, it was found lowest at the YSH-16-11 line. The reason for the low ME and NEL content in YSH-16-11 alfalfa can be explained by the fact that the CP and in vitro gas production amount of these forages are lower than other alfalfa varieties and lines.

CONCLUSION

In this study, determination of nutrient content of different alfalfa varieties and lines and determination of their in vitro GP, OMD, ME, and NEL values were the main objective. In the study, it was seen that the differences between the characteristics of alfalfa varieties and lines in terms of average GP, gas production rate, OMD, ME, and NEL were statistically significant.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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