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Determination of *In Vitro* Gas Production and Forage Qualities of Alfalfa and Sorghum-Sudangrass Forages

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Abstract: This study was conducted with the aim of determining the effects of different forage conservation methods on nutrient contents, forage quality, *in vitro* gas production and gas production parameters, energy contents and organic material digestibilities of some forages. In present study, 2 forages (alfalfa and sorghumxsudan grass) and 4 forage types (fresh, hay, silage and haylage) were used. Furthermore, grain (5%) were used in alfalfa silage and haylages and their effects on *in vitro* gas production and feed value were determined. *In vitro* gas production technique was used for determining the *in vitro* gas productions of feeds. The study was conducted by using random parcel experimental design. The highest quality was obtained in fresh form for sorghumxsudan grass and in supplemented silage form for alfalfa. While additives decreased pH values in with grain alfalfa silages and alfalfa haylages, they did not affect the organic acid contents. It was concluded that different conservation methods affected the nutrient contents and *in vitro* gas productions of forages and also that sorghum sudan grass haylages had the lowest gas production level, ME,NE_L and OMD for all the incubation times. Furthermore, there were found significant differences in terms of *in vitro* gas productions, silage qualities, feed values and nutrient contents between the silage and haylage forms of the same forage source.
Keywords: Alfalfa, fresh, gas production, hay, haylage, silage, sorghumxsudangrass

INTRODUCTION

Forages are of physiologic importance in ruminant nutrition. However, quality forage supply remains to be a significant problem. It is necessary to increase the forage cultivation areas and their yield if it is to solve this problem. Especially sorghum-sudangrass hybrid which can be cultivated in arid regions with limited irrigation opportunities offer a potential for closing this gap in quality forage supply. Sorghum-sudangrass hybrid has a high yield with increased amounts of fresh forage output per unit area (11 to 19 tons), and it is also a good secondary crop (in crop rotation) offering ease of ensilage [1-3].

Forages are conserved using a number of methods. Among these methods, the most common are drying, ensiling and haylage making. However, a number of problems occur when drying forages in some areas receiving heavy rainfall. Thus, ensiling and haylage making are important when it comes to conserving the forage. Haylage making is not as commonly used as ensiling in the world. Nevertheless, it has recently been gaining recognition due to the advantages it has to offer and scientific research is increasingly interested in exploring this method [4].Ruminants are responsible for approximately 11% (enteric fermentation) of the total methane production in the world [5].Today, animal breeders, manufacturers and research focuses on means to decrease methane production in ruminant nutrition and on forages which offer reduced methane production [6-8]. The type of forage used and their consumption levels are important factors in methane production in ruminant nutrition. It is possible to significantly reduce the greenhouse gas emissions of ruminants with feeding strategies. This study aims to define the effects of different conservation methods used for alfalfa and sorghum-sudangrass on the *in vitro* gas production, gas production parameters, metabolizable energy (ME), net energy lactation (NE_L), and organic matter digestibility (OMD) of the forage. This study is built on the hypothesis that forage conservation methods affect the nutrition values, and that ensiling and haylage making offer reduced amounts of gas production when compared to the drying method.

MATERIAL AND METHODS

Feeds supply, silage and haylage making: Silages, haylages and hay prepared using fresh alfalfa and sorghum-sudangrass hybrids were used in the experiments. Sunter variety alfalfa (n=4);Hay-Day variety sorghum x sudan grass (n=4) seeds were sown at 3 parcels of 1000 m^2 in Sakarya province of Turkey (8 m altitude,41° 0.676′ latitude (N) and 30° 34.32′ longitude (E)). In this study, fresh forages were obtained from material cut for ensiling, while hays were obtained from material cut (4 weeks after ensiling) for haylage making. Sorghum x sudangrass and alfalfa fresh materials were chopped to about 2 cm, wilted for 24 hours and then were packed into 4 replicate laboratory type PVC silos [9]. Two groups of alfalfa, one being the control group and the other being silage with 5% ground wheat, were prepared. But sorghum x sudangrass ensiled without additves. Haylages were made in 3 parallels and baled in 6 layers of nylon material. PVC pipes of 60cm x 30cm were used in haylage making. All silages and haylages were opened after two months.

Chemical analyses: Al the samples were dried in a forced-air oven at 65 °C for 48 hours. Then, dried samples were milled in a hammer mill through a 1 mm sieve for chemical analysis and *in vitro* gas production technique's assays. The samples were analyzed for dry matter, ash and crude protein (nitrogen) contents were analysed according to AOAC [10] procedure. Kjeldahl N and CP was calculated by multiplying N by 6.25. The neutral detergent fiber (NDF), acid detergent lignin (ADL), acid detergent fiber (ADF) and crude fiber (CF) analysis were done according to the method of Van Soest *et al* .[11]. using Ankom 2000 semi-automated fiber analyser. The ether extract (EE) content was determined using Ankom ^{XT15} analyzer [12]. The contents of organic material (OM), nitrogen free extract (NFE), cellulose and hemicellulose were determined by calculation.

Determining *in vitro* gas productions of forages*:* In this study, the rumen content was obtained from 2 Holstein infertile cows (average 450 kg liveweight and four years old) just now slaughtered at slaughterhouse. Rumen content mixed and it was taken under CO_2, strained through two layers of cheesecloth and was put into a thermos (39 °C) and was transported to the laboratory within 15 minutes. In this study, approximately 200 mg dry weight of samples were weighed into 100 ml calibrated glass syringes following Hohenheim gas test procedures of Menke and Steingass[13]. The syringes were warmed at 39ºC before the injection of 30 ml rumen fluid-buffer mixture (1:2) into each syringe and incubated in a water bath at 39ºC. Gas volumes were recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation. Three repetitions of each sample were used in the *in vitro* gas production experiment. Net gas productions of samples were determined at 24 h after incubation and corrected for blank and hay standard. Cumulative gas production data were fitted to the model of Ørskov and McDonald [14] by the NEWAY computer package programme:

$$y = a + b(1-\exp^{-ct})$$

where: a, gas production from the immediately soluble fraction (ml), b, gas production from the insoluble fraction (ml), a + b, potential gas production (ml), c, gas production rate constant for the insoluble fraction (ml/h), t, incubation time (h), y, gas produced at time t.

Organic matter digestibility, ME and NE_L contents of all samples were estimated using equations given below:

OMD, % = 14.88+ 0.8893GP + 0.448CP + 0.651 ash [15]

ME, MJ/kg DM = 2.20+0.136GP + 0.057CP + 0.002859 EE^2 [15]

NE_L, MJ/kg DM = 0.101GP + 0.051CP + 0.11EE [13]

Where; GP: 24 h net gas production (ml/200mg DM), CP: Crude protein (%), EE: Ether extract (%)

Determining rumen fluid pH, total volatile fatty acids and amonnia nitrogen: Rumen fluid pH values were determined using digital pH-meter in three replicates. The total volatile fatty acids and amonnia nitrogen (NH_3-N) analysis of rumen fluids were done according to Markham [16] steam distillation in three replicates.

Determination of pH and VFA analysis in silages and haylages: The pH values of samples were determined at samples obtained from different parts of silages and haylages. With this aim, 25 g sample was put in a mixer, 100 ml destile water added and mixed for 5-10 minutes. Then, the fluid part of the mix was filtered to a beaker via a filter paper and after 15-20 minutes the pH was measured using a digital pH-meter in three replicates. Volatile fatty acids contents of silages and haylages were determined by HPLC (High-Performance Liquid Chromatography, HP Agilent 1100)[17-18].

Determining relative feed values of forages: The relative feed value (RFV) of *brassica* silages were calculated as follows [19];

Dry matter digestibility (DMD, %)= 88.9-(0.779 x ADF%)

Dry matter intake (DMI, liveweight, %)= 120/(NDF%)
Relative feed value (RFV,%)= (DMDxDMI)/1.29

The quality class of the silages and haylages were determined by using Flieg score (**FS= 220+(2 x dry matter % – 15) –40 x pH**) and Total Point [20].

The required pH value in a silage is related to DM content. In other words, each silage and haylage should have a pH value which is determined according to its DM content. The "required pH values" were determined by using following formula [21]. This pH value prevents the proliferation of clostridia and enterobacteria.

Required pH (RpH)= 0.00359 x DM (g/kg) + 3.44

Statistical Analysis:

The data obtained from the experiments is analyzed using SPSS 13.0 software package Programme. Nutrient content, *in vitro* gas production, and *in vitro* true digestibility data of the feeds investigated in this study were analyzed in accordance with the completely randomized design controlling for normality and variance homogeneity. Duncan's multiple range test was used for the comparison of mean values.

RESULTS AND DISCUSSION

Nutritional contents of the forages used in the experiment are shown in Table 1. It was found that S-FRESH, S-HAY and S-SILAGE have the highest OM value, while A-SILAGE has the lowest OM value (P<0.01). The OM content (84.69%) of A-SILAGE was higher than that of reported by, while it was higher than that of (94.08%) reported by Canbolat *et al.* (2013a). It was found that the OM values found for S-HAY were in agreement with that of reported by Akdeniz *et al* [22].

Table 1. Nutrient compositions and cell wall structural elements of forages, %

FORAGE TYPE	DM	OM	CP	EE	CF	Ash	NFE	NDF	ADF	ADL	HSEL	SEL
FRESHS												
S-FRESH	34.97	93.34^{a}	7.34^{e}	2.06^{de}	24.88^{b}	6.66^{c}	59.05^{a}	53.35^{b}	29.61^{c}	6.87^{bcd}	23.74^{a}	22.75^{b}
A-FRESH	40.17	88.45^{b}	26.26^{a}	2.32^{de}	14.79^{e}	11.55^{b}	45.08^{e}	35.90^{f}	19.66^{e}	7.00^{bcd}	16.25^{b}	12.65^{e}
HAYS												
S-HAY	93.25	92.60^{a}	4.88^{f}	2.09^{de}	28.33^{a}	7.40^{c}	57.31^{ab}	56.48^{a}	31.77^{b}	4.64^{e}	24.71^{a}	27.13^{a}
A-HAY	95.27	88.41^{b}	18.88^{c}	2.76^{de}	22.49^{c}	11.59^{b}	44.28^{e}	41.29^{c}	25.63^{d}	7.61^{bc}	15.66^{b}	18.02^{cd}
SILAGES												
S-SILAGE	29.70	92.44^{a}	7.70^{e}	2.50^{de}	29.67^{a}	7.56^{c}	52.57^{cd}	57.02^{a}	32.97^{ab}	6.14^{cd}	24.04^{a}	26.83^{a}
A-SILAGE	22.14	84.69^{c}	20.26^{b}	5.65^{a}	24.94^{b}	15.31^{a}	33.85^{g}	38.71^{de}	28.85^{c}	10.10^{a}	9.86^{de}	18.75^{c}
A-SILAGE+GRAIN	31.71	89.52^{b}	17.88^{d}	4.22^{bc}	17.34^{d}	10.48^{b}	50.08^{d}	26.50^{g}	17.86^{f}	5.89^{de}	8.64^{e}	11.96^{e}
HAYLAGES												
S-HAYLAGE	39.07	92.28^{b}	7.20^{e}	1.80^{e}	29.15^{a}	7.72^{c}	54.14^{bc}	56.90^{a}	33.60^{a}	6.55^{cd}	23.30^{a}	27.04^{a}
A-HAYLAGE	41.12	88.23^{b}	17.69^{d}	3.23^{cd}	28.01^{a}	11.77^{b}	39.30^{f}	40.64^{cd}	29.04^{c}	9.75^{a}	11.59^{c}	19.30^{c}
A-AYLAGE+GRAIN	41.91	88.66^{b}	18.28^{cd}	4.92^{ab}	21.53^{c}	11.34^{b}	43.92^{e}	36.50^{ef}	25.37^{d}	8.17^{b}	11.14^{cd}	17.19^{d}
SEM		**0.76**	**0.20**	**0.37**	**0.70**	**0.76**	**1.14**	**0.77**	**0.43**	**0.45**	**0.56**	**0.42**
Significantly		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Alfalfa fresh material, S-HAY: Sorghum x sudangrass hay, A-HAY: Alfalfa hay, S-SILAGE: Sorghum x sudangrass silage, A-SILAGE: Alfalfa silage, A-SILAGE+GRAIN: Alfalfa silage suplemented with wheat, S-HAYLAGE: Sorghum x sudangrass haylage, A-HAYLAGE: Alfalfa haylage, A-HAYLAGE+GRAIN: Alfalfa haylage suplemented with wheat, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fibre, NFE: nitrogen free extracts, NDF: nötr detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, HSel:hemicellulose, Sel: cellulose . a,b,c…: Means in the same column with different letters indicate significance. a,b,c…: Means with different supercripts in the same column are significantly different. SEM=Standard error of mean.

A-FRESH was found to have the highest CP content among other forages and A-SILAGE and A-HAY come after respectively. The lowest CP value was found in S-HAY (P<0.01). The reason behind the reduced CP content in S-HAY was due to the significant loss in leaves with high protein content during the drying process. CP content of A-SILAGE (20.26%) was lower than that of reported by Faciola and Broderick [23] (24.6%). This difference may be ascribed to several factors such as the difference in subspecies, the content of the soil it is cultivated and the difference in harvest time [24]. Moreover, CP value found for A-HAYLAGE in this study was similar to that of reported by Coblentz and Walgenbach [25].

In terms of NDF, one of the cell wall fiber components, S-HAY, S-SILAGE and S-HAYLAGE gave the highest values; while A-FRESH and A-SILAGE+GRAIN gave the lowest values (P<0.01). In terms of ADF, the highest values were found from S-HAYLAGE and S-SILAGE; while the lowest was found from A-SILAGE+GRAIN. In terms of ADL, the highest values were found from A-SILAGE and A-HAYLAGE; while the lowest were found from S-HAY and A-SILAGE+GRAIN. It was shown that the use of additive (grain) decreases the NDF, ADF and ADL contents of A-SILAGE and HAYLAGE. It is known that this decrease has a positive impact on the digestibility of the forages. NDF value of A-HAYLAGE (40.64%) was found in agreement with that of reported by Coblentz and Walgenbach [25] (44.40%) while it was lower than that of reported by Hannah *et al.* [26] (51.6%). NDF values found for S-FRESH in this study were lower than that of reported by Nazli [27] (55.40%-62.85%).

Organic acid contents, pH values and the qualities of silages and haylages found for silages and haylages used in this study are shown in Table 2. In terms of pH values, S-HAYLAGE (4.35) was found to have the lowest with A-SILAGE+GRAIN (4.71) being the second lowest. In this study, it was observed that the pH value of A-SILAGE+GRAIN is statistically significantly lower than that of A-SILAGE (5.72), A-HAYLAGE+GRAIN (5.47) and A-HAYLAGE (6.17) (P<0.05). The highest pH, on the other hand, was found from A-HAYLAGE. It was also found that the pH values of A-SILAGE+GRAIN and A-HAYLAGE+GRAIN prepared using grain additive were lower than that of A-SILAGE and A-HAYLAGE without the additive. In this context, it was observed that grain addition has a positive impact on both haylage making and ensiling. Nevertheless, an important difference was found between the silages and haylages of alfalfa and sorghum sudangrass in this study (P<0.05). It is believed that this difference results from different dry matter contents, varying harvest times and different practices used in ensiling and haylage making.

Table 2. Quality class, pH and organic acids contents of silages and haylages

	S-SILAGE	A-SILAGE	A-SILAGE+GRAIN	S-HAYLAGE	A-HAYLAGE	A-HAYLAGE+GRAIN	SEM	Sig.
MpH	5.04^{d}	5.72^{b}	4.71^{e}	4.35^{f}	6.17^{a}	5.47^{c}	0.06	0.00
RpH	4.50	4.23	4.58	4.60	4.26	4.33		
Lactic acid, %	0.16^{b}	2.13^{a}	0.96^{ab}	1.41^{ab}	0.36^{b}	1.37^{ab}	0.47	0.01
Acetic acid, %	0.80^{a}	0.20^{b}	0.16^{b}	0.37^{b}	1.12^{a}	0.88^{a}	0.13	0.00
Propionic acid. %	0.52	0.20	0.04	0.00	0.17	0.12	0.18	0.12
Isobutyric acid, %	0.28^{cd}	1.08^{b}	0.75^{bcd}	0.18^{d}	0.82^{bc}	1.79^{a}	0.20	0.00
Butyric acid, %	0.56^{ab}	0.73^{a}	0.35^{ab}	0.06^{b}	0.31^{ab}	0.14^{ab}	0.19	0.09
İsovaleric acid, %	0.09^{b}	0.07^{b}	0.01^{b}	0.07^{b}	0.05^{b}	0.54^{a}	0.04	0.00
Smell	12.13	9.50	12.00	11.00	12.50	12.50	1.06	0.19
Structure	1.44	1.33	1.42	1.50	1.50	1.75	0.19	0.82
Color	3.38	3.58	3.17	3.25	3.50	3.25	0.36	0.93
Total Point	16.94	14.42	16.58	15.75	17.50	17.50	1.21	0.34
Quality Class*	Good	Good	Good	Good	Good	Good		
Flieg Point	62.45^{c}	20.61^{e}	80.22^{b}	99.35^{a}	40.33^{d}	69.66^{c}	2.56	0.06
Quality Class**	Good	Average	Exellent	Exellent	Satisfactory	Good		

MpH: Measured pH, RpH: Required pH, * Total point quality class** Flieg point quality class, a,b,c…: Means in the same column with different letters indicate significance. a,b,c…: Means with different supercripts in the same row are significantly different. SEM=Standard error of mean

While the use of additive in alfalfa silage had an insignificant effect on the organic acid content (P>0.05), it was found that the use of additive in haylages had a significant effect only on isobutyric acid and isovaleric acid (P<0.05) and wheat addition to haylages increased the organic acid content. There were significant differences between S-SILAGE and A-SILAGE in terms of lactic acid, acetic acid and isobutyric acid contents; while there was no significant difference between S-HAYLAGE and A-HAYLAGE in terms of acetic acid and isobutyric acid content (P<0.05). The use of additive in alfalfa silage significantly decreased the pH.

According to organoleptic analyses, silages and haylages were classified under GOOD quality forages, however, the classification made according to Flieg scores gave the following results: A-HAYLAGE under SATISFACTORY quality, A-SILAGE under AVERAGE quality; A-HAYLAGE+GRAIN and S-SILAGE under GOOD quality; A-SILAGE+GRAIN and S-HAYLAGE under EXELLENT quality. The quality of alfalfa silage and haylage was increased with the use of additive. In this study, the highest butyric acid value (0.73%) was found from A-SILAGE and this was detected with organoleptic analyses (smell) which in return led to the finding that A-SILAGE offers the lowest quality. Moreover, A-SILAGE also gave the lowest scores in Flieg score classification due to its low DM content and high pH value. This may be arising from low DM and low NFE contents as the lowest NFE content was found from A-SILAGE.

In silages, RpH value is suggested by Meeske [21] and each silage must have a pH value estimated according to its DM content. With the exception of S-HAYLAGE, all haylages and silages gave lower RpH values. The use of different forage species (legume-graminae), their harvest with varying DM contents and the different techniques used to ensile them (silage-haylage) are believed to be the reason behind the different RpH and MpH values obtained from the forages. Furthermore, given the value obtained for S-HAYLAGE, it is believed that RpH value will vary in silages and haylages of different forage species.

DMD, DMI and RFV values of the silages and haylages are shown in Table 3. RFV values obtained from alfalfa were consistently higher than that of sorghum-sudangrass hybrid, while their DMI and DMD values were similar. This finding is explained with the fact that alfalfa is a legume and its forage quality is higher than that of sorghum-sudangrass silages and haylages; and that alfalfa offers lower NDF and ADF contents which are used in RFV calculations.

Table 3. Forage quality class, RFV, DMD and DMI values and of forages

FORAGE TYPE	DMD,%	DMI,% LW	RFV	RFV quality class*
FRESHS				
S-FRESH	65.83^{d}	2.25^{d}	114.78^{e}	2
A-FRESH	73.59^{b}	3.35^{b}	190.94^{b}	Prime
HAYS				
S-HAY	64.15^{e}	2.13^{d}	105.66^{e}	2
A-HAY	68.94^{c}	2.91^{c}	155.34^{d}	Prime
SİLAGES				
S-SİLAGE	63.23^{ef}	2.11^{d}	103.17^{e}	2
A-SİLAGE	66.43^{d}	3.10^{c}	159.71^{d}	Prime
A-SİLAGE+GRAİN	74.99^{a}	4.53^{a}	263.50^{a}	Prime
HAYLAGES				
S-HAYLAGE	62.73^{f}	2.11^{d}	102.56^{e}	2
A-HAYLAGE	66.27^{d}	2.97^{c}	152.37^{d}	Prime
A-HAYLAGE+GRAİN	69.14^{c}	3.29^{b}	176.45^{c}	Prime
SEM	**0.34**	**0.06**	**4.05**	
Significantly	**0.00**	**0.00**	**0.00**	

DMD: Dry matter digestibility, DMI: Dry matter intake, RFV: Relative feed values, LW: Live weight, a,b,c..: Means with different supercripts in the same column are significantly different. According to the Quality Grading Standard assigned by The Hay Marketing Task Force of the American Forage and Grassland Council, the RFV were assessed as roughages based on prime >151 , 1 (premium) 151-125, 2 (good). 124-103. 3 (fair). 102-87, 4 (poor). 86-75, 5(reject).< 75. SEM=Standard error of mean

In terms of RFV, DMD and DMI of sorghum-sudangrass hybrid, S-FRESH was found to have higher values (P<0.01). In terms of RFV, the different between S-HAY and S-HAYLAGE was significant (P<0.01); while there was no significant difference between S-HAY and S-SILAGE and between S-SILAGE and S-HAYLAGE (P>0.05). It was observed that sorghum-sudangrass hybrid offers the best quality in its fresh form, while the forage conservation methods led to a decrease in forage quality.

A-SILAGE+GRAIN was found to have the highest RFV, DMD and DMI values (P<0.01). It was observed that fresh alfalfa offers higher DMD values when compared to A-HAY, A-SILAGE, A-HAYLAGE and A-HAYLAGE+GRAIN (P<0.01) Nevertheless, it was found that A-SILAGE and A-HAYLAGE offer the lowest DMD values. A-SILAGE+GRAIN gave the highest DMI value. A-SILAGE+GRAIN gave the highest RFV value among alfalfa specimens (P<0.01). According to the findings of this study, it is observed that 5% wheat addition to the alfalfa silages and haylages has a significant impact on their RFV, DMD and DMI values (P<0.01).

Table 4. *In vitro* gas productions of sorghum-sudangrass and alfalfa forages(ml/200 mg DM)

	Incubation Time, hours							
FORAGE TYPE	3	6	9	12	24	48	72	96
FRESHS								
S-FRESH	15.55^{c}	22.79^{b}	29.49^{c}	33.92^{c}	51.85^{ab}	61.78^{a}	65.77^{ab}	66.77^{ab}
A-FRESH	17.33^{abc}	25.45^{ab}	32.77^{abc}	38.01^{abc}	51.50^{ab}	56.71^{ab}	55.97^{cd}	56.55^{cd}
HAYS								
S-HAY	19.84^{ab}	27.50^{ab}	32.81^{abc}	38.12^{abc}	57.32^{a}	63.82^{a}	69.13^{ab}	70.08^{ab}
A-HAY	19.45^{ab}	27.78^{ab}	33.70^{abc}	38.88^{abc}	53.87^{ab}	58.29^{ab}	62.14^{bcd}	62.72^{bcd}
SILAGES								
S-SILAGE	16.68^{bc}	24.14^{ab}	30.52^{bc}	35.91^{bc}	54.65^{a}	59.29^{ab}	71.98^{a}	73.70^{a}
A-SILAGE	15.53^{c}	23.00^{b}	30.45^{bc}	35.75^{bc}	47.69^{b}	51.41^{b}	52.93^{d}	53.56^{d}
A-SILAGE+GRAIN	4.82^{d}	10.34^{c}	15.28^{d}	17.45^{d}	20.39^{c}	19.39^{c}	19.83^{e}	19.98^{e}
HAYLAGES								
S-HAYLAGE	2.32^{d}	4.48^{d}	6.33^{e}	7.41^{e}	13.29^{d}	17.31^{c}	20.87^{e}	21.02^{e}
A-HAYLAGE	19.97^{ab}	28.53^{a}	35.11^{ab}	40.05^{ab}	53.54^{ab}	60.74^{ab}	63.98^{abc}	64.58^{abc}
A-HAYLAGE+GRAIN	20.56^{a}	28.98^{a}	36.95^{a}	41.99^{a}	54.71^{a}	59.02^{ab}	61.10^{bcd}	61.84^{bcd}
SEM	**1.11**	**1.51**	**1.62**	**1.64**	**2.02**	**2.86**	**2.90**	**3.00**
Significantly	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

a,b,c..: Means with different supercripts in the same column are significantly different. SEM=Standard error of mean

***In vitro* gas production of forages**

The pH value of the rumen fluid used in *in vitro* gas production technique was found to be 6.62 (6.58 - 6.63); while TVFA content was 135 mmol/l (80 – 168 mmol/l) and the amount of NH_3-N was found to be 425 mg/l (320 – 530 mg/l). The rumen fluid used has properties similar to that of the standard rumen fluid [28-30]. Table 4 shows the gas production volumes of sorghum-sudangrass hybrid and alfalfa forages; gas production parameters, energy values and organic matter digestibilities of the same are shown in Table 5. As seen in the tables, S-HAYLAGE offers the lowest gas production levels throughout all incubation processes.

Table 5.Total gas production, gas production rate, ME, NE_L and OMD values of sorghum-sudangrass and alfalfa forages (ml/200 mg DM)

FORAGE TYPE	a+b, ml	c, ml/h	ME, (MJ/kg DM)	NE_L, (MJ/kg DM)	OMD, %
FRESHS					
S-FRESH	67.01^{ab}	0.06^{bc}	9.54^{b}	5.72^{b}	67.99^{b}
A-FRESH	56.86^{cd}	0.09^{b}	9.62^{b}	5.77^{b}	68.92^{b}
HAYS					
S-HAY	70.47^{ab}	0.06^{bc}	11.07^{a}	7.17^{a}	80.69^{a}
A-HAY	62.08^{bcd}	0.07^{bc}	10.63^{a}	6.91^{a}	78.23^{a}
SILAGES					
S-SILAGE	73.10^{a}	0.04^{c}	10.68^{a}	6.78^{a}	79.07^{a}
A-SILAGE	53.10^{d}	0.09^{b}	9.14^{b}	5.49^{b}	65.66^{b}
A-SILAGE+GRAIN	20.05^{e}	0.19^{a}	6.50^{c}	3.66^{c}	52.29^{c}
HAYLAGES					
S-HAYLAGE	21.88^{e}	0.04^{c}	4.44^{d}	1.95^{d}	34.33^{d}
A-HAYLAGE	64.15^{abc}	0.07^{bc}	10.74^{a}	7.07^{a}	81.54^{a}
A-HAYLAGE+GRAIN	61.18^{bcd}	0.09^{b}	10.74^{a}	6.79^{a}	79.48^{a}
SEM	**2.91**	**0.01**	**0.28**	**0.20**	**1.80**
Significantly	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

a,b,c..: Means with different supercripts in the same column are significantly different. SEM=Standard error of mean

S-HAYLAGE was found to have the lowest value when the 24-hours incubation values obtained from alfalfa and sorghum-sudangrass hybrid considered. S-HAYLAGE and A-HAYLAGE+GRAIN showed relatively significantly lower gas production (P<0.01). This can be explained with the low pH values of S-HAYLAGE and A-HAYLAGE+GRAIN (low pH value is associated with reduced gas production). In addition, low gas production of S-HAYLAGE may also be associated with the differences in ensiling and haylage making (delayed harvest time, nitrogen free extract contents, differences in ensiling technology used, etc.). The gas production estimated for the 24-hours incubation of A-HAY (53.87 ml/200mg DM) was higher than that of reported by Abas *et al.* [31] (31.29-52.54 ml/200mg DM), Kamalak [32] (52.67 ml/200mg DM), Kilic [33] (40.7 ml/200mg DM), Polat *et al.* [34] (29.57-33.64 ml/200mg DM), Canbolat and Karaman [35] (44.6-52.9 ml/200mg DM) and Canbolat *et al* [36] (51.70 ml/200 mg DM); while it was lower than that of reported by Aydın [37] (56 ml /200mg DM). The gas production estimated for the 24-hours incubation of A-SILAGE (47.69 ml/200mg DM) was lower than that of reported by Kamalak [32] (56.33 ml/200mg DM) and Canbolat *et al.* [38] (52.43 ml/200mg DM); while it was higher than that of reported by Muck *et al* [39] (29.8-33.74 ml/200mg DM) and Saricicek and Kilic [40] (36.12 ml/200mg DM).

Also known as the total gas production, the “a+b” value was highest for S-SILAGE (73.10 ml); while it was the lowest for A-SILAGE+GRAIN (20.05 ml). A-SILAGE+GRAIN and S-HAYLAGE gave relatively significantly lower values when compared to the other forages (P<0.01). In terms of the gas production rate, the “c” value, A-SILAGE+GRAIN gave the highest value; while there was a significant difference between A-SILAGE+GRAIN and the other forages (P<0.01). As grain addition reduces the pH level in the rumen, the fact that it may increase the acidosis risk must be considered.

A closer look into Table 5 showed that S-HAYLAGE gives the lowest values in terms of ME, NE_L and OMD (P<0.01) with A-SILAGE+GRAIN (4.71) being the second lowest. There were no statistically significant differences between S-HAY, S-SILAGE, A-HAY, A-HAYLAGE and A-HAYLAGE+GRAIN, and S-FRESH, A-FRESH and A-SILAGE. As 24-hours *in vitro* gas production values are used in order to calculate these values, ME, NE_L and OMD values of those with high gas production were also high. The ME value found from A-HAY in this study (10.63 MJ/kg DM) was higher than that of reported by Canbolat and Karaman [35] (9.3-10.5

MJ/kg DM); while it was lower than that of reported by Canbolat *et al* [36] (10.88 MJ/kg DM).

It is believed that consideration must be given to available resources and economic conditions in the determination of the forage conservation method for different forages. In addition, building on the fact that straws, hay, and even silages (bales silage) are being traded today in order to meet the forage needs, it was observed that haylage is an alternative forage conservation method which can be turned to by stockfarmers in any time of the year. Among the alfalfa forages used in this study, A-SILAGE+GRAIN showed to have the best properties and it was found that the best conservation method for alfalfa is to ensile it using 5% wheat additive. A closer look into the nutrition value and forage quality of sorghum-sudangrass hybrid, a forage which can be cultivated under severe weather conditions with high yield, showed that it is an important forage source for stockfarming and that the amount cultivated must be increased as sorghum-sudangrass hybrid can be cultivated as the second crop and it grows in a relatively short period. It is believed that sorghum-sudangrass hybrid is an important forage source for arid and semi-arid regions with limited quality forage supply.

CONCLUSIONS

In conclusion, it was observed that the use of wheat additive (5%) in alfalfa ensiling and haylage making has a significant impact on the end product, while reducing the gas production. Indeed, reduced *in vitro* gas production means that the methane production will also be reduced. In this context, its contribution to economical stockfarming is obvious both in terms of environmental footprint and wasted feed energy. Consumption of A-SILAGE+GRAIN which is found to have the lowest gas production among alfalfa forages and S-HAYLAGE which is found to have the lowest gas production among sorghum-sudangrass forages in this study as a forage source may contribute to the measures taken in order to reduce the greenhouse gas emissions originating from stockfarming.

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