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Research Article

The Effect of Various Additives Addition to Barley-Vetch Mixtures on Silage Quality Characteristics and *In vitro* Digestibility

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Abstract
In this study, it was aimed to determine the effects of adding different levels of molasses and fructose syrup to barley-vetch mixtures, one of the roughage sources produced as a second product in cotton fields in Şanlıurfa province, on some silage quality characteristics and ruminal methane gas formation by *in vitro* gas production technique. In the study, the additive-free silage group constituted the control group, while the silages, prepared by adding different levels of fructose syrup (0.1%, 0.2%, 0.3%, 0.4%) and molasses (0.5%, 1%, 1.5%, and 2%) to the control group, formed the treatment groups. In the study, while *in vitro* organic matter digestion (IVOMD) and metabolic energy (ME) values of silages increased in all experimental groups compared to the control group, CH_4 gas production decreased. The lowest pH value among the silage groups was obtained from the silage prepared with the addition of 1.5% and 2% molasses. It was observed that the ammonia nitrogen (NH_3-N/TN) value of the silages decreased in the silage prepared by adding 1.5% molasses. While the highest amount of CO_2 was determined in the control group, the lowest value was determined in the silage group with 1.5% molasses added. The highest value in terms of lactic acid content was determined in the control group, while the lowest was determined in the silage group with 0.5% molasses. When the acetic acid contents of the silages were examined, it was seen that all additives decreased the acetic acid contents of the silages compared to the control silage. As a result, it was concluded that the addition of 1.5% molasses or 0.4% fructose syrup additive had positive effects on silage fermentation, improved *in vitro* organic matter digestion and metabolic energy values, and could be used as it was economical and applicable.

Keywords
Legumes, grains, *in vitro*, silage

INTRODUCTION

The use of annual forage crops as a winter intermediate product is important in terms of protecting the soil from erosion during the empty period between the productions of two main crops, suppressing weeds, and providing organic matter to the next plant. In Turkey, roughage production is quite insufficient according to the presence of farm animals (Yolcu and Tan, 2008). Dry grass production, which can be described as high quality in Turkey, is around four million tons. When silage production is considered, only 30-35% of the roughage demand in Turkey is provided (Ozkan, 2020). Intermediate forage plants production within the vacant winter season production season has the characteristics of being quality roughage in closing our roughage deficit, which is around 60%. Harvest times of plants are important for growing mixed silage material. Vetch must be harvested in full bloom to make silage from grain mixes (Avcı and Ayaşan, 2007). Leguminous fodder crops are rich in protein, but are difficult to ensilage on their own. Grain forage crops, on the other hand, are good in terms of easily digestible carbohydrate content, but are insufficient in terms of protein. For this reason, the silage of the plants belonging to these two families by mixing them in appropriate proportions will allow to obtain quality roughage (Demirel et al., 2010). It is essential to use additives to support fermentation during the ensiling of difficult-to-ensilage forage crops. For this purpose, it is frequently utilized from additives such as molasses, cereal grains, fruit pulp, sugar, etc. to meet the WSC needs (Broderick et al., 2002). In this study, it was aimed to determine the effects of adding different levels of molasses and fructose syrup to barley-vetch mixtures, one of the roughage sources produced as a second product in Şanlıurfa province, on some silage quality characteristics and ruminal methane gas formation by *in vitro* gas production technique.

MATERIAL and METHODS

In the study, a mixture of barley-vetch was used as silage raw material. The silage raw material was obtained from the field of a farmer cultivating roughage in Şanlıurfa, by shredding it with 5-7 cm silo track. Barley (*Hordeum vulgare*) and vetch (*Vicia sativa L.)* were planted together at the rate of 1:1. Barley-vetch mixtures were harvested at milk stages of the barley and chopped. The lactic acid bacteria numbers of barley and vetch plants used in the study were determined according to lactic acid bacteria MRS Agar pour plate technique (De Man et al., 1960). Buffering capacity (BC) determinations of barley and vetch plants were performed according to the method reported by Playne and McDonald (1966), and WSC contents were determined in reference to the method reported by Dubois et al. (1956). A total of 9 different silage groups were formed by adding 0.1%, 0.2%, 0.3%, 0.4% fructose syrup and 0.5%, 1%, 1.5% and 2% molasses to the silage groups prepared in the study, control (without additives) according to the wet weight base. In order to ensure homogenization in all silage groups prepared in the research, 10 ml/kg distilled water was added to all groups. Each trial group of silages was compressed into 1.5 liter glass jars with 5 repetitions, and they were ensiled up in an airtight manner. Silages were stored at room temperature for 60 days in a dark environment. After the silages were opened at the end of the 60-day fermentation period and the 3-5 cm part at the top of the jars was discarded, 100 ml of distilled water was added to the homogeneously taken 25 g silage sample and shredded for 2 minutes with the help of a blender, and the pH value of the shredded silage liquid was recorded by measuring with pH meter (WTW 7310) measuring device (Polan et al., 1998). The liquid in the blender was filtered and taken into 10 ml tubes; 0.1 ml of 1M HCl was added on the samples to be analyzed for ammonia nitrogen and 25% 0. 25 ml of meta phosphoric acid was added on the samples to be analyzed for lactic acid and volatile fatty acid and stored in a deep freezer until analysis. The ammonia nitrogen ratio (NH_3-N/TN, %) values in the total nitrogen (TN) content of the silages obtained was performed according to the method reported by AOAC (1990), and lactic acid and volatile fatty acids (butyric, acetic and propionic acid) concentrations were determined using a high pressure liquid chromatography device (HPLC) according to the method reported by Suzuki and Lund (1980). For this purpose, it was utilized from high performance liquid chromatography (HPLC) device (Shimadzu LC-20 AD HPLC pump, shimadzu SIL-20 ADHT Autosampler, Shimadzu SPD M20A Detector (DAD), Shimadzu cto-20ac Colum oven, Icsep Coregel (87H3 colon). The aerobic stability values of the silages obtained in the study were determined in reference to the method reported by Ashbell et al. (1991). The raw nutrient contents such as the dry matter, raw ash and crude protein analyzes of the silages obtained from vetch-barley mixture used as silage material in the study were performed according to AOAC (2005), while ADF and NDF analyzes were performed according to Van Soest et al. (1991). The raw nutrient analyzes were carried out after the silage materials and the obtained silages were dried at room temperature and then ground in a laboratory mill to pass through a 1 mm sieve. The *in vitro* organic matter digestibility (IVOMD) and metabolic energy (ME) contents of the silages obtained in the study were determined according to the method reported by Menke (1988). The data obtained from current study were evaluated with One Way Analysis of Variance (One Way Anova). Duncan multiple comparison tests were used to compare group means and for this purpose, SPSS (1991) package program was used.

RESULTS

The nutrient contents of barley and vetch used as silage material in the study are given in Table 1. lactic acid bacteria (LAB), buffering capacity (BC), water soluble carbohydrates (WSC), dry matter (DM), crude ash (CA), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), *in vitro* organic matter digestibility (IVOMD), and metabolic energy (ME) values of barley and vetch plants used as silage material in the study were determined as $4x10^6$ cfu/g, 312 meq/kg DM, 28.24 g/kg DM, 30.30%, 9.73%, 11.60%, 34.80%, 58.77%, 55.97%, and 8.29%, respectively, according to dry matter basis.

Table 1. Raw nutrient contents of fresh material (barley-vetch mixture) used in the study

LAB	BC	WSC	DM	CA	CP	ADF	NDF	IVOMD	ME
$4x10^6$	312	28.24	30.3	9.73	11.60	34.80	58.77	55.97	8.29

LAB: Lactic acid bacteria cfu/g; **BC**:Buffering capacity meq/kgKM; **WSC**: Water-soluble carbohydrates g/kg DM, **DM**: Dry matter, DM %; **CA:** Crude ash DM%; **CP**: Crude protein, DM%; **ADF**: Acid detergent insoluble fiber, %DM; **NDF**: Neutral detergent insoluble fiber, %DM; **IVOMD**: *In Vitro* organic matter digestion, **ME**: Metabolic energy

Nutrient content of silages prepared by adding fructose syrup (0.1%, 0.2%, 0.3%, 0.4%) and molasses (0.5%, 1%, 1.5%, 2%) to barley and vetch plants used as silage material in the study are given in Table 2. It was determined that the additive application was statistically significant ($p<0.05$) in terms of the dry matter (DM) rate, which is one of the properties examined. It is seen that the DM rate varies between 23.29-26.00%. Due to the increase in the amount of molasses used as an additive in this study, the DM values of the silages obtained especially with the addition of 2% molasses increased compared to the control and 2 and 3 % fructose syrup groups. While the crude ash (CA) values were at the highest level in the 0.2% fructose syrup group, they were the lowest in the 0.1% fructose syrup and 1.5% molasses group ($p<0.05$). In the study, crude protein (CP) value of silages prepared with the addition of fructose syrup and molasses additives at different levels were found to be higher in the silage group prepared with 1% molasses addition compared to the control and other groups. When the ADF and NDF values of the silages were examined, it was seen that the ADF value increased due to the increase in fructose syrup compared to the control group, while it decreased due to the increase in molasses addition ($p<0.05$). While the NDF value was at the highest level in the group prepared with 0.2% fructose syrup addition compared to the control group, the NDF value decreased due to the increase in molasses ($p<0.05$). In the study, *in vitro* organic matter digestion and metabolic energy values of barley and vetch added silages prepared by adding different levels of fructose and molasses additives were found to be higher than the values obtained from the control silage when compared with the additive-free silage ($p<0.05$). The highest *in vitro* organic matter digestion and metabolic energy values were obtained from the 2% molasses added silage group, while the lowest *in vitro* organic matter digestion and metabolic energy values were obtained from the control silage. When the % CH_4 values of the silages were examined, it was seen that they were at the highest level in the control group, but decreased due to the increase in fructose syrup addition ($p>0.05$).

Table 2. Nutrient content of silages prepared by adding various additives to legume - grain forage mixtures (%)*

Groups	DM	CA	CP	ADF	NDF	IVOMD	ME	CH_4
Control	23.76^{e}	10.06^{b}	11.89	34.99^{c}	58.71^{ab}	55.00^{e}	8.14^{e}	13.69
0.1% Fructose Syrup	23.61^{f}	9.87^{b}	12.19	36.02^{ab}	58.58^{ab}	55.24^{e}	8.17^{e}	13.24
0.2% Fructose Syrup	23.29^{h}	11.03^{a}	11.80	36.30^{ab}	58.84^{a}	55.99^{de}	8.28^{de}	13.04
0.3% Fructose Syrup	23.29^{h}	9.97^{b}	11.94	36.30^{ab}	58.41^{ab}	57.34^{cd}	8.36^{cde}	12.98
0.4% Fructose Syrup	23.52^{g}	9.97^{b}	11.62	36.07^{ab}	58.48^{ab}	57.85^{bc}	8.57^{bc}	12.72
0.5% Molasses	23.85^{d}	10.34^{ab}	12.28	36.66^{a}	58.21^{bc}	57.22^{cd}	8.47^{cd}	12.95
1% Molasses	24.03^{c}	10.33^{ab}	12.54	35.72^{b}	57.66^{c}	57.83^{bc}	8.54^{bcd}	13.30
1.5% Molasses	25.27^{b}	9.60^{b}	12.28	33.50^{d}	57.67^{c}	59.16^{ab}	8.78^{ab}	13.23
2% Molasses	26.00^{a}	10.08^{b}	12.49	33.48^{d}	55.75^{d}	60.26^{a}	8.94^{a}	13.50
SEM	0.17	0.09	0.08	0.20	0.16	0.31	0.04	0.07
P Value	**0.000**	**0.016**	0.058	**0.000**	**0.000**	**0.000**	**0.000**	0.090

***:** Values with different letters in the same column were found to be different ($P<0.05$); **DM:** Dry matter, %; **CA:** Crude ash DM%; **CP:** Crude protein, DM%; **ADF:** Acid detergent insoluble fiber, %DM; **NDF:** Neutral detergent insoluble fiber, %DM; **IVOMD:** *In Vitro* organic matter digestion, **ME:** Metabolic energy, **CH_4:** *In Vitro* methane gas (ml/g); **SEM:** standard error mean

Fermentation characteristics of silages prepared by adding fructose syrup (0.1%, 0.2%, 0.3%, 0.4%) and molasses (0.5%, 1%, 1.5%, 2%) to barley and vetch plants used as silage material in the study are given in Table 3. When the pH value of the silages prepared by adding additives were examined, it was determined that the highest 0.2% fructose syrup (3.99) and the lowest pH value (3.76) were obtained from the silages prepared with the addition of 1.5% and 2% molasses ($p<0.05$). When the ammonia nitrogen value of the silages was examined, it was seen that the highest value (8.31) was obtained from 0.2% fructose syrup compared to the control group and decreased due to the molasses addition, but the lowest was obtained from the silages with 1.5% molasses. The CO_2 production amounts of the silages varied between (2.74-7.15 g/kg DM). The highest CO_2 value (7.15) was determined in the control group. Lactic acid level, which is one of the fermentation criteria, was found to be at a low level compared to the control group. The highest lactic acid content (40.26 g/kg) was found in the control group, while the lowest was in 0.5% molasses (7.99 g/kg) group. When the acetic acid contents of the silages were examined, decreases were observed in the acetic acid contents of the silages to which various additives were added compared to the control silage ($P<0.05$). The highest acetic acid content (7.79 g/kg) was obtained from the control group, while the lowest acetic acid content (1.65 g/kg) was obtained from the 0.5% molasses group. When the LA/AA ratios of the silages were examined, it was seen that the highest LA/AA ratio (5.17) was obtained from the control group silage, while the lowest LA/AA ratio (3.53) was obtained from 1% molasses added silage ($p<0.05$).

Table 3. The effect of silages prepared by adding various additives to legume-grain forage plant mixtures on fermentation characteristics*

	pH	NH_3N/TN	CO_2	LA	AA	LA/AA
Control	3.83^{bc}	7.77^{ab}	7.15^{a}	40.26^{a}	7.79^{a}	5.17^{a}
0.1% Fructose Syrup	3.87^{b}	7.13^{bc}	3.36^{b}	18.57^{b}	4.75^{b}	3.91^{e}
0.2% Fructose Syrup	3.99^{a}	8.31^{a}	3.90^{b}	8.53^{f}	2.10^{g}	4.07^{de}
0.3% Fructose Syrup	3.88^{b}	7.43^{bc}	2.77^{b}	15.86^{d}	3.78^{e}	4.20^{cd}
0.4% Fructose Syrup	3.86^{bc}	6.84^{cd}	3.49^{b}	8.23^{g}	2.16^{g}	3.82^{e}
0.5% Molasses	3.86^{bc}	7.28^{bc}	3.33^{b}	7.99^{h}	1.65^{h}	4.83^{b}
1% Molasses	3.79^{bc}	6.85^{cd}	3.21^{b}	9.05^{e}	2.57^{f}	3.53^{f}
1.5% Molasses	3.76^{c}	6.28^{d}	2.74^{b}	18.47^{b}	4.28^{c}	4.32 ^{cd}
2% Molasses	3.76^{c}	6.38^{d}	3.38^{b}	17.94^{c}	4.09^{d}	4.39^{c}
SEM	0.01	0. 12	0. 24	1.92	0.35	0.09
P Value	**0.010**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

***:** Values with different letters in the same column were found to be different ($P<0.05$); **NH_3-N/TN:** Ammonia nitrogen; **CO_2:** Carbon dioxide; **LA:** Lactic acid; **AA:** Acetic acid; **SEM:** standard error mean

DISCUSSION and CONCLUSION

The LAB content of the barley and vetch plant used in the study was determined as $4x10^6$ cfu/g, and the BC was 312 meq/kg DM. The source of lactic acid bacteria is the epiphytic flora on the plant material, and its main source is the soil. The LAB density on the plant varies according to many factors such as the dry matter content of the plant, the development period of the plant, the presence of nutrients on the leaves, soil characteristics, altitude and climatic conditions. The WSC content of the barley and vetch plant used in the study was determined as 28.24 g/kg DM. Canbolat et al. (2013), in their study, found the WSC content of fresh alfalfa plant as 1.38. This difference is due to the fact that the alfalfa plant is high in protein and low in carbohydrates. Kendall (1978) stated that legume grasses naturally contained insufficient amount of WSC, and that vegetation period, harvest method, weather conditions and fertilizer use during production were effective on the WSC content of plants in general. Similarly, Haigh (1990) reported that the WSC content in silage plants was affected by climatic conditions, and the WSC content of plants cultivated in regions with low sunlight and high precipitation were low. The dry matter contents of the silages obtained by adding fructose syrup (0.1%, 0.2%, 0.3%, 0.4%) and molasses (0.5%, 1%, 1.5%, 2%) to the mixtures of barley and vetch at different rates were found in the range of (23.29-26.00). Considering the DM contents, it is thought that the increase in their content may be due to the high DM content of molasses added to the silage. The literature reports that molasses increase the DM content also support these findings (Arslan et al., 2020; Bingöl and Baytok, 2003). The ADF and NDF contents of the silages prepared in this study were found to be significantly lower in the 2% molasses added group compared to the other groups ($P<0.05$). Li et al. (2014) reported that the addition of sucrose, glucose, molasses and cellulase to *Peucedanum ostruthium* silages reduced the ADF and NDF contents of the silage compared to the control group. It can be said that the reason for the difference in the findings about ADF and NDF is the plant species used in the trials, harvest periods, mixing ratios and ecological conditions (Turan, 2019; Turan and Seydoşoğlu, 2020; Özyazıcı et al., 2022). Carr et al. (2004) stated that the proportion of the substances forming the cell wall decreased depending on the proportional increase in the intracellular content. In our study, the highest protein ratio in molasses 1.5-2% and the lowest NDF ratio in molasses 1.5-2% compared to the other groups are consistent with the report of Carr et al. (2004). The IVOMD, ME contents of the silages increased with the addition of 0.4% fructose syrup and molasses (0.5%, 1%, 1.5% and 2%) compared to the control group. The CH_4 contents of the silages decreased in all trial groups compared to the control group. There are reports that silages prepared by adding molasses increase the IVOMD and ME values, and molasses increases the digestibility values by increasing the disintegration of ADF and NDF. Li et al. (2014) reported that the addition of sucrose, glucose and molasses and cellulase enzyme to *Peucedanum ostruthium* silage and Arslan et al. (2020) reported that the addition of molasses to grass silage increased the IVOMD and ME contents of silages. These reports support the results obtained from this study. The lowest pH value of the silages obtained by adding fructose syrup (0.1%, 0.2%, 0.3%, 0.4%) and molasses (0.5%, 1%, 1.5%, 2%) to the mixtures of barley and vetch at different rates were obtained from the silage with 1.5% and 2% molasses. It was reported that the most suitable pH range for the development of LAB in the acid environment of silage pH was 3.8-4.2 (Ergün et al., 2013). Arslan et al. (2020) reported that adding molasses to grass silage reduced the pH value of silages. In the study conducted by Turan (2019), the pH value of the silage obtained by mixing Hungarian vetch and barley wet grass at different ratios was similar to the present study, while it differed from the pH value of the silage obtained by mixing oats, barley, rye, triticale and vetch at different ratios in the study conducted by Görü and Seydoşoğlu (2021). In this study, it was determined that the NH_3-N value of silage increased with 0.2% fructose syrup addition compared to the control group, and decreased in silages with 1.5% and 2% molasses addition ($p<0.05$). In the study, it was reported that molasses additive reduced proteolysis by making a positive effect on silage fermentation (Bingöl et al., 2009). Compared to the control group, the CO_2 production amount of the silages decreased the CO_2 amount in all groups with additives. It is known that ferments in the silage environment in the aerobic period intensively produce CO_2. Homolactic fermentation in the additive groups is lower than in the control group. When low homolactic fermentation silages are exposed to oxygen, the metabolites produced as a result of heterolactic LAB fermentation have an inhibitory effect against microorganisms that cause silage spoilage, prevent the growth and activity of yeasts, reduce CO_2 production, that is, increase aerobic stability values (Ali et al., 2020). The highest lactic acid content of the silages was determined in the control group ($p<0.05$). When the acetic acid content of the silages was examined, a decrease in the acetic acid content of the silages with various additives was observed compared to the control silage ($p<0.05$). When the LA/AA ratios of the silages were examined, the highest LA/AA ratio was obtained from the control group silage, while the lowest LA/AA ratio was obtained from the silages with 1% molasses ($p<0.05$). It is reported that when the LA/AA value in silage is greater than 3.0, homolactic fermentation occurs, and if it is less than 3.0, heterolactic fermentation occurs (Zhang et al., 2010). Since all values were greater than 3 in this study, homofermentative lactic acid bacterial fermentation was thought to be dominant in all groups. Besides, the highest lactic acid value and LA/AA ratio were determined in the control group. This showed that the highest homofermentative lactic acid bacterial fermentation was in the control group. The low LA and LA/AA ratio in all groups compared to the control group causes a decrease in the lactic acid value in silage by not degrading the lactic acid of the heterofermentative LAB (Danner et al., 2003; Taylor et al., 2002).

As a result, it was concluded that the addition of 1.5% molasses or 0.4% fructose syrup had positive effects on silage fermentation, *in vitro* organic matter digestion improved and metabolic energy values and it can be used as it is economical and applicable.

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