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Araştırma Makalesi / Research Article

Effects of Different Silage Additives on Silage Quality of Gramineae Forage Mixtures

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Abstract

There should be no tables, figures or bibliography. In this study, silage quality properties, in vitro organic matter digestion (IVOMS), metabolic energy (ME) and in vitro methane gas values were investigated when different silage additives were added to barley, triticale and ryegrass forage crop mixtures at different mixing ratios grown as winter catch crop in Adıyaman province of Turkey. In the study, control group silages was not recieved any additives, while treatment groups silages prepared by addition of homofermentative lactic acid bacteria, heterofermentative lactic acid bacteria, 0.2% molasses and 0.2% fructose. In the study, IVOMS, ME and in vitro CH4 values of silages were found to be similar ($p>0.05$). While the lowest pH value was determined in the control group among the silage groups, the highest value was obtained from the silage prepared with the addition of 0.2% fructose. It was observed that the ammonia nitrogen (NH3-N/TN) value of the silages were increased with homofermentative lactic acid bacteria addition and decreased with addition of 0.2% molasses ($p=0.000$). The highest amount of CO2 was detected in the control group, while the lowest value was determined in the silage group to which homofermentative lactic acid bacteria were added ($p=0.00$). 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 0.2% fructose. When the acetic acid contents of the silages were examined, it was observed that all additives decreased the acetic acid contents of the silages compared to the control silage ($P=0.001$).

Keywords: Fructose, heterofermentative, homofermentative, molasses, silage

INTRODUCTION

Meeting the quality, cheap and regular roughage requirement is the most important problem to be solved for the development of Turkey's livestock production sector. In addition to suitability of roughage utilisation to animal feeding physiology, high quality and cheap roughage will decrease the concentrated feed requirement, which is more expensive (Özkan, 2019). Roughage such as green grass, dry roughage and silage feeds increase profitability of animal production enterprices as they do not burden in terms of cost (Alçiçek et al., 1995; Bilgen et al., 1996). In silage production, it is important to produce alternative silage materials to maize crop to prepare silage. Due to the difficulties experienced in the preparation of silages of leguminous forage crops, which are rich in protein (high buffering capacity, low fermentation quality, etc.), some meadow grasses with carbohydrate-rich content can be grown as a mixture with grasses. Thus, a silo feed rich in energy, crude protein and mineral substances can be obtained. In recent years, ryegrass production has started in Turkey, especially in the Marmara, Aegean and Mediterranean regions, where the climate and soil conditions are available. The ryegrass species, which is mostly given to ruminants as fresh after mown or fed by grazing, is also used by making hay or silage (Özkul et al., 2012). It was determined that silage of cut Ryegrass in the form of bale silage and haylage has similar values, bale silage is better than hay in terms of feed efficiency, and there is loss of value in terms of protein and energy when stored as hay (Mc Cormick et al., 1998). The use of barley, triticale and ryegrass as silage is not very common yet. Mixed cropping of barley, triticale and ryegrass in fallow fields and their utilization as silage may be one of the important alternatives in eliminating the quality forage deficit. In this study, it was aimed to determine the effects of barley, triticale and ryegrass forage crop mixtures grown as winter catch crop in Adıyaman (Turkey) on some silage quality properties, IVOMS, ME and *in vitro* methane gas formation by *in vitro* gas production technique.

MATERIAL and METHODS

Study design and silage preparation

Mixtures of barley, triticale and ryegrass (50%, 25%, 25%) plants were used as silage raw material in the study. The silage material was obtained from the field of a farmer who cultivates roughage in Adıyaman province and obtains 35-40 tons of fresh silage per hectare, and was obtained by shredding it with the help of a 5-8 cm silo truck. In order to ensure homogenization in all prepared silage groups, 10 ml/kg distilled water was added to the silages. The total lactic acid bacteria (LAB) count in the fresh silage material was determined by the method reported by Güney and Ertürk (2020) as three replications for each group according to the tempo automatic bacterial counter test method. The buffering capacity (BC) of fresh barley, triticale and ryegrass used in the study was determined according to the method reported by Playne and McDonald (1996). Barley, triticale, ryegrass mixtures prepared without additives in the study constituted the control group, while silages prepared by adding homofermentative lactic acid bacteria ((Pioneer®, USA) ($1x10^6$ cfu/gr)), heterofermentative lactic acid bacteria ((Pioneer®, USA) ($1x10^6$ cfu/gr)), 0.2% molasses (w/w) and 0.2% (w/w) fructose formed the treatment groups. Used homofermentative lactic acid bacteria were included *Lactobacillus plantarum* DSM 18112, *Lactobacillus plantarum* DSM 18113,

Lactobacillus plantarum DSM 18114, *Lactobacillus plantarum* ATCC 55943, *Enterococcus faecium* ATCC 55593, *Enterococcus faecium* ATCC 53519 strains. Used heterofermentative lactic acid bacteria were included *Lactobacillus buncheri* ATCC PTA-2494 strain. Each trial group of silages was compressed into 1.5 liter glass jars with five replications, and were siled up in an airtight manner. Silages were stored at room temperature for 60 days in a dark environment.

Fermentation profile analysis

The silages were opened at the end of the 60-day fermentation period, then 3-5 cm of the top part of the jars was discarded, 100 ml of distilled water was added to the homogeneously taken 25 g silage sample and shredded for two minutes with the help of a blender, the pH value of the crushed silage liquid was rapidly measured with a pH meter (WTW 7310) (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 to the samples to be analyzed for ammonia nitrogen, and 0.25 ml of 25% metaphosphoric acid was added to the samples to be analyzed for lactic acid and volatile fatty acid. and stored in deep freezer until analysis. According to the method reported by AOAC (1990), the ammonia nitrogen ratio (NH_3-N/TN, %) values in the total nitrogen (TN) content of the silages obtained; 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, high performance liquid chromatography (HPLC) device (Shimadzu L.C-20 AD HPLC pump, shimadzu SIL-20 ADHT Autosampler, Shimadzu SPD M20A Detector (DAD), Shimadzu cto-20ac Columum oven, Icsep Coregel (87H3 colon)) was used. The aerobic stability values of the obtained silages were made according to the method reported by Ashbell et al. (1991). The dry matter (DM), ash, and crude protein (CP) analyzes of the silages obtained with barley, triticale, ryegrass used as silage material in the study were conducted according to AOAC (1990); ADF and NDF analyzes were performed according to Van Soest et al. (1991). 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 (Şimşek Laborteknik) to pass through a 1 mm sieve. The digestibility (IVOMS), ME and *in vitro* CH_4 contents of the silages obtained in the study were determined according to the method reported by Menke et al. (1988), with five replications for each sample.

Statistical analysis

The data obtained at the end of the research were evaluated with one-way analysis of variance (OneWay Anova). Duncan multiple comparison test was used to compare group means. For this purpose, SPSS (1991) package program was used. Level of significance was taken as $P<0.05$.

RESULTS

The nutrient contents of barley, triticale and ryegrass mixtures (BTR) used as silage material in the research are given in Table 1. The total number of LAB, BC, DM, ash, CP, ADF, NDF, IVOMS, and ME values of the BTR mixture plants used as silage material in the study were were determined as 4.107 cfu/gr, 260meq kg/DM, 34.01%, 6.75%, 7.74%, 36.82%, 49.26 and 7.32%, respectively.

Table 1. Raw nutrient contents of barley, triticale, ryegrass mixtures used in the study

	Total LAB Count	BC	DM	Ash	CP	ADF	NDF	OMS	ME
BTR	4.10^7 kob/g	260	34.01	6.75	7.74	36.82	60.64	49.26	7.32

BTR: mixture of barley, triticale and ryegrass, BC: Buffering capacity (meq kg/DM), DM: Dry matter, %; Ash, DM%; CP: Crude protein, DM%; ADF: Acid detergent insoluble fiber, %DM; NDF: Neutral detergent insoluble fiber, %DM; IVOMS: *In Vitro* Organic matter digestion, ME: Metabolic Energy

The nutrient contents and IVOMS, ME, and *in vitro* CH_4 values of silages prepared by adding homofermentative and heterofermentative lactic acid bacteria, fructose and molasses to barley, triticale and ryegrass mixtures used as silage material in the study are given in Table 2.

Table 2. Nutrient content and IVOMS, ME and CH_4 values of silages prepared by adding various additives to cereal forage plant mixtures

Grups	DM	Ash	CP	ADF	NDF	IVOMS	ME	CH_4 ml/g
Control	41.16^b	6.84	8.44	37.06^a	62.45^a	50.02	7.43	22.94
Heterofermentative LAB	40.15^d	6.90	8.40	37.54^a	61.72^b	51.58	7.57	25.64
Homofermentative LAB	40.54^c	6.74	8.37	37.52^a	62.39^a	49.41	7.22	24.12
0.2% Molasses	44.12^a	6.62	8.16	36.45^b	61.44^b	50.27	7.47	25.24
0.2% Fructose	41.42^b	6.90	8.47	37.39^a	61.34^b	50.55	7.50	25.64
SEM	0.288	0.038	0.052	0.10	0.108	0.34	0.061	0.48
P Value	0.00	0.089	0.407	0.001	0.000	0.407	0.465	0.322

$^{a,b,c,d:}$ Values with different letters in the same column were found to be different (P<0.05); DM: Dry matter, CP: Crude protein, ADF: Acid detergent insoluble fiber, % DM; NDF: Neutral detergent insoluble fiber, % DM, IVOMS: *In Vitro* Organic matter digestion, ME: Metabolic Energy (MJ/kg DM), CH_4: *In* Vitro methane gas (ml/g).

When Table 2 is examined, DM values of silages obtained by addition of 0.2% molasses increased compared to the control group, while DM values decreased with the addition of homofermentative and heterofermentative LAB additives (P=0.00). In the study, the ash and CP values of the silages prepared with the addition of various additives was not changed (P>0.05). ADF values were found to be lower (P=0.001) with the addition of 0.2% molasses, and lower than the value obtained from the control group silage with the addition of heterofermentative, 0.2% molasses and 0.2% fructose (P=0.00). In the study, IVOMS and ME and *in vitro* CH_4 values of silages obtained by adding various additives were found to be similar when compared with silage without additives (P>0.05). Fermentation characteristics of silages prepared by adding homofermentative and heterofermentative LAB, fructose and molasses to barley, triticale and ryegrass forage plants used as silage material in the research are given in Table 3.

Table 3. Fermentation characteristics of silages prepared by adding various additives to cereal feed plant mixtures

Grups	pH	NH_3 N/TN	CO_2	LA	AA	LA/AA
Control	3.73^{c}	5.53^{ab}	5.19^{a}	27.84^{a}	9.80^{a}	2.84 ^{a}
Heterofermentative LAB	3.83^{b}	5.22^{b}	1.37^{c}	20.75^{b}	9.24^{b}	2.24 ^{c}
Homofermentative LAB	3.80^{b}	5.60^{a}	1.36^{c}	18.65^{b}	6.29^{d}	2.97 ^{a}
0.2% Molasses	3.81^{b}	4.38^{c}	1.40^{c}	18.89^{b}	7.45^{c}	2.54 ^{b}
0.2% Fructose	3.86^{a}	5.27^{ab}	2.42^{b}	7.22^{c}	2.61^{e}	2.76 ^{ab}
SEM	0.00	0.99	0.30	1.62	0.60	0.07
P Value	0.000	0.000	0.000	0.000	0.000	0.000

$^{a,b,c,d:}$ 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 g/kg DM, LA: Lactic acid g/kg DM, AA: Acetic acid g/kg DM.

In the study, the lowest pH value (3.73) of the silages prepared with various additives was determined in the control group, and the highest pH value (3.86) was obtained from the silage prepared with the addition of 0.2% fructose. The CO_2 production amounts of the silages varied between 1.36-5.19 g/kg DM. The highest CO_2 value (5.19 g/kg DM) was determined in the control group. When the NH_3 N/TN values of the silages were examined, it was seen that homofermentative LAB added silages increased compared to control silages and decreased in silages prepared with 0.2% molasses addition (p=0.000). In terms of lactic acid value, which is one of the fermentation criteria, the LA content of the added silages was found to be low. The highest lactic acid content (27.84 g/kg DM) was found in the control group, while the lowest (7.22 g/kg DM) was found in the 0.2% fructose group. When the acetic acid contents of the silages were examined, it was observed that the acetic acid values of the silages with all additives were reduced compared to the control silage (P=0.001). The highest acetic acid content (9.8 g/kg DM) was obtained in the control group, and the lowest acetic acid value (2.61 g/kg DM) was obtained from the 0.2% fructose group. When the LA/AA ratios of the silages were examined, the highest LA/AA ratio (2.84) was obtained from the control group silage, while the lowest LA/AA value (2.24) was obtained from heterofermentative LAB added silages (p=0.000).

DISCUSSION and CONCLUSION

The DM contents of silages prepared by adding homofermentative, heterofermentative LAB, molasses and fructose to mixtures of barley, triticale and ryegrass at different rates were found to be in the range of 40.15-44.12%. Considering the DM contents, it was observed that the DM contents increased significantly in the silage group (0.2%) with molasses addition compared to the control group. The increase in the DM content of the silage groups prepared by molasses addition may be due to the high DM content of the molasses added to the silage. The literature regarding the addition of molasses resulting with increase in DM content of silages also supports these findings (Bingöl and Baytok 2003; Bingöl et al.,2009;Seydoşoğlu and Gelir 2019a; Seydoşoğlu 2019b; Seydoşoğlu and Gelir 2019). The ADF and NDF contents of the silages prepared in this study were found to be significantly lower in the molasses added group compared to the control group ($p<0.05$). This decrease is attributed to the decrease in the amount of ADF and NDF in the silage by increasing the amount of lactic acid bacteria, which is one of the anaerobic

bacteria of molasses added to the silage by Bolsen et al., (1996). On the other hand, Bingöl et al.,(2003) stated in their study that 6% molasses addittion to barley and sainfoin mixed silage significantly reduced silage ADF and NDF values compared to the non-added group, and this decrease was due to the low ADF and NDF content of molasses (Bingöl et al; 2009). These reports support the results obtained from this study. IVOMS, ME and *in vitro* CH_4 contents of the silages were found to be similar to the control group ($p>0.05$). Bingöl et al., reported that the silages prepared by adding different levels of molasses to the barley and sainfoin mixture had significantly higher digestibility compared to the control silage. Tabioka et al., (1991) reported that molasses additive increased the digestibility values of silage in a study they conducted by addition of molasses to barley. They attributed the increase of IVOMS and ME values of silages prepared by adding molasses, to the degradation of ADF and NDF and thus to the increase of digestibility values of molasses. The lowest pH value (3.73) of the silages obtained by adding homofermentative LAB, heterofermentative LAB, molasses and fructose to mixtures of barley, triticale and ryegrass in different ratios was determined in the control group, while the highest pH value (3.86) was obtained from silage prepared by adding 0.2% fructose. Filya et al. reported that the pH value was between 4.5, 3.8 and 3.8 in the control, LAB and LAB+enzyme groups, respectively, in a study where they examined the effects of LAB and LAB+enzyme inoculants on the sorghum plant they harvested and ensiled during the milk dough period (Filya et al., 2001). It was reported by Ergün et al. that, the pH value of silage is one of the most important factors affecting silage fermentation, the most suitable pH range for the development of LAB that grows in an acid environment is 3.8-4.2, and bacteria that cause deterioration and decay can not survive in silage with a value in this pH range (Ergün et al., 2013). In this study, it was determined that silage NH_3-N value increased in homofermentative LAB added silages and decreased in silages prepared with 0.2% molasses addition ($p=0.000$). In the study, it is observed that molasses additive has a positive effect on silage fermentation and reduces proteolysis. Bingöl et al. determined that the NH_3-N value of the silages they prepared by adding 4% and 6% molasses to the barley forgae and sainfoin mixture decreased, which supports the results obtained in this study (Bingöl et al.,2009). Dolezal et al.(2005) reported that the addition of 5% and 7% molasses to lupine silage increased the silage fermentation quality and decreased NH_3-N values. Carpintero et al. (1979), reported that the silage %NH_3-N/TN value should be lower than 11% in order for silages to be evaluated in the good quality silage class. The amount and composition of pH, NH_3-N and organic acids (acetic acid, butic and lactic acid) formed during silage fermentation determine the quality of fermentation. Especially silages with low pH and NH_3-N amounts and high lactic acid/acetic acid ratios can be considered as well-fermented silages (Filya et al., 2001). It is known that yeasts in the silage environment in the aerobic period intensively produce CO_2. In this study, the CO_2 values of the silages prepared by adding various additives were found to be low. This can be explained by the fact that the dominant LAB in the doped groups produces metabolites that inhibit the proliferation of aerobic bacteria and yeasts, especially in the silage medium. In this study, the differences between the

groups in terms of IVOMS and ME and *in vitro* CH_4 values of silages obtained by adding various additives were not found statistically significant when compared to silage without additives. Güler et al. (2019), reported that probiotics added to maize silage had no effect on *in vitro* organic matter digestion and methane gas production, which supports the current study (Güler et al. 2019). In terms of silage lactic acid value, which is a fermentation criteria, the highest lactic acid content was found in the control group, while the lowest value was determined in the 0.2% fructose group. When the acetic acid contents of the silages were examined, a decrease was observed in the acetic acid contents of all silages with added additives compared to the control silage. The highest acetic acid value was obtained from the control group, and the lowest acetic acid value was obtained from the 0.2% fructose group. When LA/AA was examined, the highest LA/AA value was obtained from the control group silage, while the lowest LA/AA value was obtained from the silage supplemented with heterofermentative lactic acid bacteria. Homolactic fermentation is reported when the LA/AA ratio in silage is greater than 3.0, and heterolactic fermentation occurs when the LA/AA ratio is less than 3.0 (Zhang et al., 2015). In this study, the lowest LA/AA ratio was observed in the heterofermentative LAB supplemented group, and the highest LA/AA ratio in the homofermentative LAB supplemented groups, which was in agreement with the literature reports. Compared with the control group, the low LA/AA ratio in heterofermentative and molasses additives indicates that heterolactic LAB fermentation occurs in silages. When silages are exposed to oxygen, the amount of acetic acid produced by heterolactic LAB fermentation or enterobacteria increases and has an inhibitory effect against microorganisms that cause silage to deteriorate. It also prevents the growth and activity of yeasts, reducing CO_2 production and improving aerobic stability values (Ali et al., 2020). In addition, when heterofermentative LAB formed in barley-triticale and ryegrass mixtures are examined in terms of acetic acid and LA/AA values in this study, it is thought that they evaluate molasses additive better than fructose, with high acetic acid and low LA/AA ratio. As a result, it was concluded that 0.2% molasses additive used in barley, triticale and rygress mixed silages reduced proteolysis by lowering the ammonia nitrogen of the silages, and improved aerobic stability by reducing the amount of CO_2.

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