EFFECTS OF MOLASSES, BACTERIAL INOCULANT AND ENZYME + BACTERIAL INOCULANT ADDITION ON SILAGE CHARACTERISTICS, IN VITRO ORGANIC MATTER DIGESTIBILITY AND METABOLISABLE ENERGY CONTENT OF GRASS SILAGE

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Abstract. This study was carried out to evaluate molasses, microbial inoculant and microbial inoculant + enzyme (MICROBIOS) *(Lactobacillus plantarum, Lactobacillus brevis, Propionibacterium shermanii, Enterococcus faecium, Bacillus subtilis, Pediococcus acidilactici* and alpha–Amylase (*A. oryzae*), cellulase and hemicellulose (*A. niger*)) addition as silage additives on nutrient contents, in vitro organic matter digestibility (IVOMD) and metablisable energy (ME) of grass silage. The material mixed with additive was pressed in (1.0-1 L) glass jars. Each application consisted of three parallel. Three jars per treatment from all group were analayzed on day 2, 7, 21, 60 for chemical, in vitro digestibility organic matter, metebolisable energy and cell wall contents. According to the analysis; control, molasses, enzyme + inoculant and inoculant groups of dry matter (DM) 26.59, 26.47, 27.00, 26.65, pH 4.75, 4.38, 4.29, 4.04 were found. Additives (molasses, microbial inoculant, enzyme + microbial inoculant) were able to ensure fermentation quality. Particularly inoculant and inoculant + enzyme improved the digestibility organic matter and metabolisable energy contents of silage.
Keywords: *grass, silage additive, feed value, cell wall, minerals*

Introduction

Silage is the main forms of preserved grass and other forages for livestock in Europe and North America (Randby et al., 2015). Pasture grasses is moderately suitable to ensiling due to their botanical composition and low water-soluble carbohydrate contents (Gul et al., 2008; Yuksel, 2019; Arslan et al., 2020). In order to improve feeding value and silage preservation different additives (such as: bacterial inoculant, molasses, enzyme, grains etc.) have been applied (Keady, 2000). Molasses which are the rich sugars and fermentable carbohydrate contents and are also easily handled all over the world. Molasses improved silage fermentation characteristics such as pH and lactic acid concentration (Baytok and Muruz, 2003; Burenook et al., 2012). Enzymes have been used as additives either alone or in combination with lactic acid bacteria (LAB). Enzymes show hemiselluloytic and celluloytic activites. Thus, these activities solubilize the cell wall carbohydrates, increasing the substrate availability for LAB, and after all improve the silage fermentation quality (McDonald et al., 1991; Rinne et al., 2020). Bacterial inoculants contain one or more type of homofermentative LAB that are fast and efficient of lactic acid. The main purpose of using homofermentative LAB inoculants is to improve the nutritional value and to reduce the risk of clostridial fermentations (Driehuis et al., 2001; Muck et al., 2017).

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This study was carried out to evaluate the effects molasses, microbial inoculants and enzyme + microbial inoculants as silage additives on nutrient contents, in vitro organic matter digestibility and metabolizable energy value of grass silage.

Material and methods

The study was conducted in Tekirdag (41.0°N, 27.5°E), western Turkey located at about 5 m altitude above sea level and with a total precipitation of 482 mm on average and an annual mean temperature of 10.5 °C. Proportions of the Gramineae, Leguminoseae and other plant families in the pasture grasses were 50.3-51.0%, 31.3-34.8% and 14.2-18.4% of the flora, respectively (Altin et al., 2010). Forage was chopped (1.0-1.5 cm theoretical length of cut). Silage materials were divided into four trial groups for the control, molasses, inoculant and enzyme + inoculant treatments. (1) The chopped forage treatment control; (2) treatment mollases; applied at rate of 5% of fresh forage. (3) inoculant, a mixture of lactic acid bacteria (LAB) consisting of Lactobacillus plantarum and Enterococcus faecium applied at a rate of 6.00 log10 cfu LAB·g-1 of fresh forage (Pioneer 1188, USA). (4) Treatment enzyme + inoculant: enzym as, a mixture of enzymes consisting of cellulase, amylase, hemicellulase and pentosanase enzymes applied at a rate of 0.01 mg·g-1 of fresh forage (Enzyme, Global Nutritech 41600 Kandira, Kocaeli-Turkey), On the day of the experiment, molasses, inoculants and enzymes were suspended in 10 ml of tap water and the whole suspension was sprayed over 5 kg (wet weight) of the chopped forage spread over a 1 × 4 m area. All additives were applied to the forages in a uniform manner with constant mixing (Ozduven et al., 2009, 2010). The material mixed with additive was pressed in (1.0-1 L) glass jars (Weck, Wher-Oftlingen, Germany) equipped with lids that enabled gas release only. The jars were stored under constant room temprature (20 ± 1 °C). Three jars per treatment from all group were sampled on day 2, 7, 21, 60 for analayses of chemical, cell wall contents, in vitro organic matter digestibility and metabolisable energy contents of grass silages.

Analytical procedure

Chemical analyses were performed on triplicate samples. The fresh and silage samples were dried at 60 °C for 72 h in a fanassisted oven. After drying samples were ground through a 1 mm screen for chemical analysis. The dry matter (DM) was determined by drying the samples at 105 °C for 4 h. Crude protein and ash contents of samples were determined according to the methods of AOAC (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) content determined as described by Van Soest et al. (1991). Metabolisable eenergy (ME) content of fresh and silage samples were calculated from the chemical composition Anonymous (1991). In vitro OMD contents of silages were determined according to the enzyme method reported by Naumann and Bassler (1993). For this purpose, pepsin enzyme (Merck, 0.7 FIP-U/g, Germany) and cellulase enzyme obtained from Trichoderma viride microorganisms (Merck, Onozuka R10; Germany) were used. Ammonia nitrogen (NH_3-N) and pH values fresh and silage samples were determined according to Anonymous (1986). Lactic acid (LA) was determined by the spectrophotometric (Shimadzu UV_12 ol, Kyoto Japan) method Barker and Summerson (1941). Fermentation losses during storage were estimated by weight loss, calculated separately for each jar by the difference in the weight at the beginning and end of the ensiling period. Ca and P content of samples were determined to the methods of AOAC (1990).

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Statistical analysis

Statistical analyses were performed with the general linear model (GLM) procedure of Duncan's multiple range test performed with the Statistical Analysis System. Software (SAS, Cary, NC).

$$Y_{ijk} = \mu + a_i + b_j + a_{bij} + e_{ijk} \quad \text{(Eq.1)}$$

Y_{ij} = studied traits; μ = overall mean; $a_{i\ =}$ = effect of b factor; e_{ij} = error; b_j = effect of b factor; ($a_{bij)\ (a*b)}$ = interaction effect.

For all statistical comparisons, a probability level of $P < 0.05$ was accepted as statistically significant. When significant associations were identified, the mean values for each effect were contrasted using Duncan test.

Results

Nutrient content of the silage is presented in *Table 1*. It was determined that the effects of the applications on the DM contents on the 7th and 60th days of the silages were insignificant. The DM contents on the 2nd and 21st days of the silages were determined as 28.43-29.92%, 24.50%-26.03%, respectively, and the difference between the treatments was statistically significant ($P < 0.05$).

CP contents of the silages were determined with the lowest 6.61% DM in the inoculant application on the 21st day, while the highest value was detected on the 21st day with the molasses application with 7.71% DM. The differences between the applications were found to be statistically significant ($P < 0.05$).

The lowest pH value was found with 4.04 in the inoculant group on the 60th day, the highest pH value with 5.39 on the 2nd day of the control group. When the pH contents of the silages were evaluated, the differences between the applications were found to be statistically significant ($P < 0.01$).

LA and WSC contents of silages were determined in the range of 3.29%-3.54% DM, 5.50%-28.50% g/kg DM in all treatment groups, and the differences between treatment groups were statistically insignificant.

The lowest NH_3-N contents of the study were found in the control group on the 7th day with 75.68% g/kgTN and the highest in the control group on the 2nd day with 83.54 g/kgTN. Differences between the 21st and 60th day treatment groups were found to be statistically significant ($P < 0.05$) (*Table 2*).

NDF, ADF and ADL contents of silages were determined in the range of 58.72%-60.22% DM, 44.55%-45.60% DM, 9.23%-10.16% DM in all treatment groups. and the differences between treatment groups were statistically insignificant (*Table 3*).

In vitro organic matter digestion (IVOMD) and metabolic energy (ME) contents of grass silages were determined and given in *Table 4*. In the study OMD value ranged between 49.85%-58.72% respectively. The highest OMD was determined as 58.72% in the inoculants + enzyme group ($P < 0.01$).

ME contents of silages ranged between 1.42%-1.71MJ/kg DM respectively. The highest ME contents was determined as 1.71% MJ/kg DM in the inoculants group ($P < 0.01$) (*Table 4*).

Ca and P contents of silages were determined and given in *Table 5*. In the study P value ranged between 0.26%-0.30% respectively. The highest P contents was determined as 0.30% in the inoculants group ($P < 0.01$).

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Table 1. *Results of the chemical analyses of the grass silages*

Day	Treatment	DM %	Weight loss %	pH	CP %(DM)	CF %(DM)
2	C	28.76 b	0.54 b	5.39 a	7.46	31.50 b
	M	28.43 b	0.65 b	5.02 b	7.27	28.58 ab
	E + I	28.60 b	0.86 a	4.40 d	7.12	32.81 c
	I	29.92 a	0.54 b	4.47 c	7.33	33.42 a
	SEM	0.238	0.050	0.153	0.063	0.721
	P	0.033	0.040	0.000	0.316	0.004
7	C	28.45	0.66 ab	5.17 a	7.50 a	34.05 a
	M	28.81	0.59 b	4.89 b	7.63 a	30.51 c
	E + I	28.52	0.75 a	4.06 d	6.78 b	31.82 b
	I	28.59	0.58 b	4.53 c	6.85 b	32.90 ab
	SEM	0.108	0.028	0.156	0.151	0.507
	P	0.763	0.043	0.000	0.021	0.005
21	C	26.03 a	0.51 b	4.65 a	7.66 a	32.97 a
	M	24.50 b	0.56 ab	4.57 b	7.71 a	31.15 b
	E + I	25.50 ab	0.60 ab	4.20 d	7.07 ab	32.36 ab
	I	25.48 ab	0.64 a	4.50 c	6.61 b	32.78 ab
	SEM	0.226	0.020	0.064	0.184	0.312
	P	0.070	0.078	0.000	0.029	0.119
60	C	26.59	0.55 ab	4.75 a	6.99	33.43
	M	26.47	0.59 ab	4.38 b	7.14	32.19
	E + I	27.00	0.51 b	4.29 c	6.77	33.32
	I	26.65	0.63 a	4.04 d	6.90	33.16
	SEM	0.112	0.186	0.097	0.073	0.242
	P	0.465	0.054	0.000	0.386	0.279

$P < 0.05$, $P < 0.01$
DM: dry matter, CP: crude protein, CF: crude fiber, C: control, M: molasses, I: inoculant, E + I: enzyme + inoculant

Discussion

In this study addition of molasses, inoculant, and enzyme + inoculants were significantly affected DM contents (2nd and 21st days) of the silages (*Table 1*) ($P < 0.05$). The higher DM contents in the silages might be related to the readily additives. Additives (molasses, inoculant, and enzyme + inoculants) improve the fermentation and thus preventing the undesirable fermentation of silage and DM loses.

Silage dry matter content is similar to the findings of Bureenok et al. (2012), Khota et al. (2016), Ofori and Nartey (2018); Rinne et al. (2020). It was found to be lower than the findings of Gul et al. (2008), Vendramini et al. (2016), Randby et al. (2015) and Arslan et al. (2020). The difference between the DM findings of the study and the literature findings is due to the plant composition, soil structure and the different additives used.

For good silage fermentation aerobic requirements and reduced pH should be ensured. The pH value usually drops through the fermentation of lactic acid Van Soest (1994). Inoculant + enzyme, inoculant and molasses, added silage groups showed a

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significant decrease in pH value compared to the control group (*Table 1*) ($P < 0.01$). The lowest pH value was obtained on day 60th with the addition of inoculant.

Table 2. *Results of the chemical analyses of the grass silages*

Day	Treatment	NH3-N g/kgTN	LA %(DM)	WSC g/kgDM
2	C	83.54	3.29	28.00
	M	77.44	3.31	27.00
	E + I	80.67	3.33	28.50
	I	79.18	3.38	25.00
	SEM	1.044	0.016	1.042
	P	0.195	0.325	0.754
7	K	75.68	3.37	19.50
	M	78.39	3.40	18.00
	E + I	77.55	3.32	20.50
	I	79.68	3.35	18.50
	SEM	0.667	0.014	0.895
	P	0.173	0.335	0,.850
21	C	79.13 a	3.40	13.00
	M	77.43 bc	3.40	11.50
	E + I	78.84 ab	3.42	9.50
	I	76.64 c	3.43	10.50
	SEM	0.414	0.012	0.789
	P	0.034	0.923	0.829
60	K	76.20 b	3.54	7.50
	M	78.37 a	3.52	5.50
	E + I	76.50 b	3.51	6.50
	I	78.83 a	3.51	6.50
	SEM	0.43	0.012	0.626
	P	0.030	0.846	0.827

$P < 0.05$, $P < 0.01$
WSC: water soluble carbohydrates, LA: lactic acid, NH3-N: ammonia nitrogen, C: control, M: molasses I: inoculant, E + I: enzyme + inoculant

Table 3. *Cell wall contents of the grass silages (% DM)*

Day	Treatment	NDF	ADF	ADL
60	C	60.20	44.82	9.83
	M	58.72	45.35	9.23
	E + I	59.90	44.55	10.00
	I	60.22	45.60	10.16
	SEM	0.343	0.225	0.207
	P	0.440	0.384	0.481

NDF: nötral detergan fiber, ADF: acid detergen fiber, ADL: acid detergen lignin, C: control, M: molasses, I: inoculant, E + I: enzyme + inoculant

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Table 4. *In vitro OMD and ME contents of grass silage*

Day	Treatment	IVOMD %	ME MJ kg DM
60	C	53.34 bc	1.58 b
	M	49.85 c	1.42 c
	E + I	58.72 a	1.69 ab
	I	56.14 b	1.71 a
	SEM	1.307	0.045
	P	0.016	0.005

$P < 0.05$, $P < 0.01$
OMD: organic matter digestibility, ME: metabolize energy, C: control, M: molasses, I: inoculant, E + I: enzyme + inoculant

Table 5. *Mineral matter contents of grass silage (%)*

Day	Treatment	P	Ca
60	C	0.29 ab	0.54
	M	0.26 b	0.53
	E + I	0.26 b	0.45
	I	0.30 a	0.54
	SEM	0.071	0.025
	P	0.000	0.630

$P < 0.05$, $P < 0.01$
C: control, M: molasses, I: inoculant, E + I: enzyme + inoculant

The pH values of the silages are similar to the study findings used as additives such as arion vulgaris (Randby et al., 2015), *Lactobacillus buchneri* (Driehuis et al., 2001), fibrolytic enzyme (Rinne et al., 2020) and molasses (Vendramini et al., 2010). At the same time, the pH findings of the study, which used Lactic acid + acetic acid (Vendramini et al., 2016), cassava foilage (Mao et al., 2018) and enzyme as additives (Arslan et al., 2020) were found to be lower than the study findings used, but higher than the study findings using lactic acid and molasses (Bureenok et al., 2012) as additives. The difference between the study findings and previous study findings is due to the additives used and the plant composition.

In this study it was emphasized that use of silage additives induced a decrease CP contents of treatment groups as compared to control group. The highest CP content was found in the molasses group day of 21st.

The research findings were lower than the findings of Khota et al. (2016) who use cellulose and inoculant as additives, Baba et al. (2018) who use corn, soy, molasses and Arslan et al. (2020) who use molasses, oak tannins barley. The difference between research findings and previous study findings is due to the plant composition, soil structure and additives used.

NH_3-N content should not exceed 100 g/kg total nitrogen (Van Soest et al., 1991). All the treatments silage groups met these criteria. This study result emphasized that additives unchanged NH_3-N concentration as compared to control groups. The highest NH_3-N contents found in control group day of 2nd. In accordance with our silage results Arslan et al. (2020) indicated that use of 25 g/kg molasses addition unchanged NH_3-N concentration of silage.

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In this study the highest OMD and ME contents were established in enzyme + inoculant silage group. Our study results accordance with Kaya et al. (2009 a) emphasized 40 g/kg barley or 20 g/kg molasses addition increase the organic matter digestion. Another study conducted by Kaya et al. (2009b) was found that 25 g/kg and 50 g/kg barley addition to grass silage did not affect organic matter digestion. Arslan et al. (2020) results indicated that oak tannin extracts, previously fermented juice (OTE and PFE) addition decreased OMD and ME values. Difference may be based on variety, additives and used different in vitro methods.

Ca and P contents of the study are given in *Table 5*. The highest P content was detected in inoculant application.

The additives used did not change the Ca content of the silages. The highest Ca content was found in the control and inoculant group. Tomaz et al. (2018) emphasized that Ca and P content did not change in their study using inoculant as an additive. The reason why the research findings are lower than those of Tomaz et al. (2018) is due to the amount of Ca and P contained in meadow grass and soil.

Conclusion

As a result of the study, it was determined that use of inoculant, molasses, enzyme + inoculant as silage additive improved the nutrient contents and silage fermentation quality. In vitro organic matter digestion and metabolic energy values of grass silage molasses followed the effect of inoculant.

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