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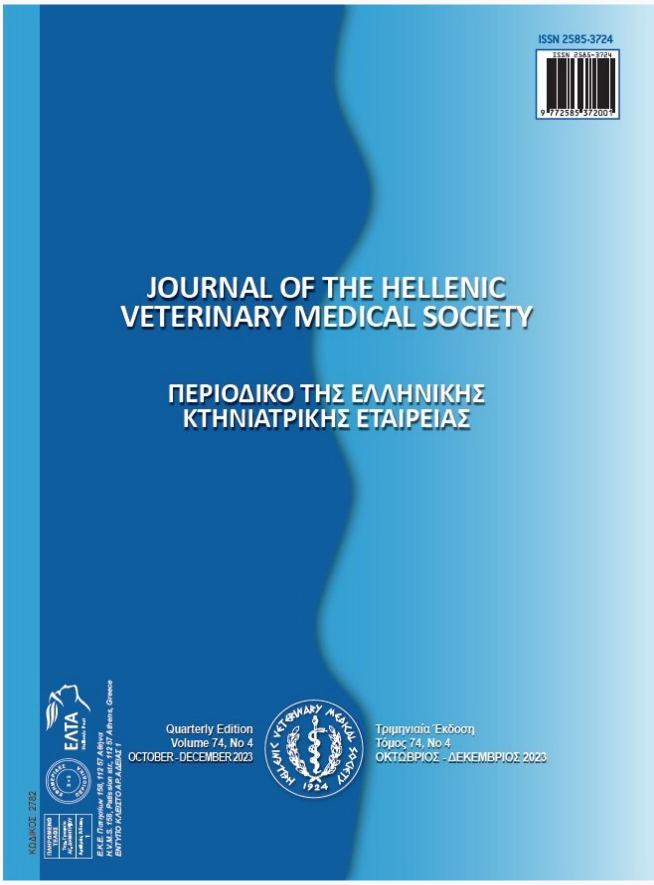

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The effects of adding molasses and inoculant to silages of fodder pea and rye grass in different proportions on silage quality

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ABSTRACT: The aim of this study was to determine the effects of adding melas and microbial inoculant to the mixed silages of different proportions fodder pea (FP) and rye grass (RG) grown in arid conditions on silage quality and *in vitro* digestibility. For this purpose, silages containing fodder pea and rye grass at 20, 40, 60, and 80% ratios were prepared in jars with additives 5% molasses and 10 g/ton inoculant (1.25×10^{11} CFU/g) and waited 60 days. At the end of the study, a significant difference was determined in the pH value, lactic acid (LA), acetic acid (AA), and ammonia-N levels among groups of the mixed silages ($P<0.05$). It was determined that inoculant was effective on the LA level ($P<0.05$). The interaction between mixture level and additive was observed at pH value, LA, and ammonia-N levels ($P<0.05$). *In vitro* digestibility, energy values, and nutrient contents of silages showed significant change among groups ($P<0.05$). While molasses significantly increased the dry matter levels, it decreased the acid detergent fiber (ADF) level ($P<0.05$). The interaction between mixture level and additive was observed at only neutral detergent fiber (NDF) level ($P<0.05$). While the structure was positively affected in silages containing 80% FP, the Flieg score decreased in silages containing 60% FP ($P<0.05$). With addition of molasses increased the Flieg score of silages, and it showed interaction between mixture level and additive ($P<0.05$).As a result, although mixed silages containing 80% FP had high ammonia-N, excellent fermentation was observed with low pH value and high LA level. In addition, although NDF and ADF levels increased, mixed silages with FP at 80% levels have higher *in vitro* digestibility and energy levels. Each of the additives had a positive effect on silages, but molasses was determined to be more effective.

Keywords: Fodder pea;inoculant; molasses;rye grass; silage quality

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INTRODUCTION

In addition to providing species diversity and balanced roughage for animals, pastures are important in terms of being a cheap source in countries with high animal production costs (Ayan et al., 2020). However, the inability to reach enough pasture grasses in the dry seasons of the year requires compensation with grain stubble. These products, which have insufficient nutritional content, also cause a decrease in animal productivity. For these periods when nutritious feedstuffs are insufficient, quality feed sources should be stored. In addition, it is desirable that quality feeds being offered to animals should have a balanced energy protein content. This is possible with the mixed planting of legumes and graminae compatible with the harvest time (Seydoşoğlu, 2019a).

The nutritional material that is formed as a result of fermentation by storing the feeds containing soluble carbohydrates in its structure under airless conditions and having various end products is called silage (Kunget al., 2018).Roughages can be evaluated as hay or silage. Some factors affect the choice between these evaluation methods. Climatic conditions are considered among the most important of these. While the drying method is preferred in arid regions with less precipitation, the silage method is preferred in regions with abundant precipitation (Kurtoğlu, 2011). However, feed quality decreases significantly due to climatic conditions and other processes during the drying time. Silage production is important in terms of access to quality rough and moisture feed, which is insufficient for all times of the year. For an ideal fermentation, it is important that there be soluble and easily digestible carbohydrates in the silage environment. When ensiling feed materials with low, easily digestible carbohydrates, additives rich in digestible carbohydrates may be needed. Also, additives used during ensiling are added to improve beneficial fermentation and prevent undesirable fermentation(Acar and Bostan, 2016; Tekin and Kara, 2020; Nascimento et al., 2023).Along with its rich soluble carbohydrate content, 250-400 g/kg dry matter (DM) level and low buffer capacity are required features for a good silage material. Silage dry matter level has a strong effect on fermentation. Low dry matter and sugar levels will cause clostridial fermentation and adversely affect the consumption of animals (Nikosi and Meske, 2010). In addition, silage fermentation and quality can be evaluated by pH, organic acid production, nitrogen composition, and organoleptic evaluations. As a matter of fact, volatile structures released as the end product of fermentation can cause different odors (Kunget al., 2018). Digestibility is one of the important criteria that shows the quality of a feed. There may be differences in digestibility even between different varieties of the same species of feeds. In the study conducted by Boga and Ayaşan (2022) with different varieties and lines of alfalfa, significant differences were determined between organic matter digestibility and metabolizable energy values. The authors stated that this was due to the variety and line types used, the time of slaughter, and the fact that the samples were taken from different places. In addition, because it is expensive, difficult, and takes too much time to predict the digestibility of feed using live animals in ruminants, the in vitro digestion method has been developed (Tassone et al., 2020).

The aim of this study was to determine the effects on silage quality and in vitro digestibility of adding melas and microbial inoculants to the mixed silages of different proportions fodder pea (FP) and rye grass (RG) grown in arid conditions.

MATERIALS AND METHODS

The study was carried out in arid conditions of Central Anatolia and with plants grown without irrigation and artificial fertilizers. The experimental area was in the of Kırıkkale University campus in Kırıkkale province in the Central Anatolian region of Türkiye (39°53'04.9" N, 33°26'20.0" E). The annual precipitation amount of the region,which has hot and dry summer months and cold and rainy winter months, is 405 mm;however, it was 414.3 mm in the year of the study. The soil of the study area is slightly alkaline (pH = 7.73),salt-free (0.10 EC (dS/m)), moderately calcareous (12.15%), and has a low phosphorus (3.13 ppm) level. The soil organic matter level of the study area is low (1.33%). While it has a sufficient level of potassium (216 ppm),the nitrogen level (0.18%) is also low. These data were obtained as a result of analyses. Rye grass (RG) (*Lolium multiflorum L.*) and fodder pea (FP) (*Pisum sativum var. arvense*) species were planted in 5 × 1.5 m^2 plots as 3 replications. RG and FP seeds were sown as 5 rows with a 15 cm distance in each plot. The amount of seeds sowed in RG and FP species was 6 kg/da and 15 kg/da, respectively. RG and FP seeds were sown at 20, 40, 60, and 80% ratios in each plot. At the end of the growing process of the plants, manual harvesting was carried out from a 1 m^2 area of each plot. Approximately 10 kg of samples were freshly taken from the harvested area at each mix ratio and chopped into approximately 2-3 cm lengths. The chopped samples were laid on an area of approximately 2 m^2 and 5% molasses and,10 g/ton inoculant (1.25×10^{11} CFU/g) were applied. Inoculant used was obtained from DuPont Pioneer Company, 1188 silage inoculant® that contains; (*Lactobacillus plantarum* LP286 DSM 4784 ATCC 53187 : 2.5×10^{10} CFU/g, *Lactobacillus plantarum* LP318 DSM 4785: 2.5×10^{10} CFU/g, *Lactobacillus plantarum* LP319 DSM 4786 : 2.5×10^{10} CFU/g, *Lactobacillus plantarum* LP346 DSM 4787 ATCC 55943 : 2.5×10^{10} CFU/g, *Enterococcus faecium* SF301 DSM 4789 ATCC 55593 : 1.25×10^{10} CFU/g, *Enterococcus faecium* SF202 DSM 4788 ATCC 53519 : 1.25×10^{10} CFU/g). After the additive applications, the samples were mixed homogeneously. Then, each one of 4 different ratios of HV and FP were manually compressed into a total of 48 jars of 1.5 L in 4 replications as control, molasses, and inoculant groups. After the lids were tightly closed, the jars were left to stand at 20-25 ℃ for 60 days. At the end of 60 days, all the jars were opened, and their fermentation parameters, physical properties, chemical components, *in vitro* digestibility, and energy values of the silages were determined.

Opened silages were scored according to*German Agricultural Organization (*DLG, 1987) in terms of odor, color, and structure. According to the total score obtained, the quality evaluation of the silages was scored as: Very good (18-20), Good (14-17), Intermediate (10-13), Low (5-9) Deteriorated (0-4). Then, chemical analyses were carried out. For this aim, 100 mL of distilled water was added to 25 g silage samples taken from each opened jar and mixed homogeneously by means of a mixer. The liquid part of the mixture was filtered. pH values, ammonia-N concentrations,and organic acid levels were measured from the filtered liquid. The pH measurement was made with a digital electronic pH device (HANNA, HI 2221). Ammonia-N concentrations were determined by the Kjeldahl distillation (VELP® UDK 129) method according to Filya (2003). Some of the filtrate was stored at -20°C until analysis of organic acids. The lactic acid (LA) concentration in the filtrate was determined according to Tekin and Kara (2020) with a modified method by Barnett (1951). Other organic acid concentrations (butyric acid (BA), propionic acid (PA), and acetic acid (AA)) were also determined according to Tekin and Kara (2020).

After physical property scoring of opened silages and sampling for fermentation parameters, the remaining silages were left to pre-dry with air. Then, to determine the dry matter and chemical components, the sample was taken and dried in an oven at 65 °C for 72 hours. After drying, these samples were ground through a 1 mm screen in the mill to analyze other components and results were given as dry matter (DM). The crude protein (CP) and ash levels of silage samples were determined according to the methods reported by the Association of Official Agricultural Chemists (AOAC) (2005). Organic matter (OM) levels were determined as the remaining value after subtracting the ash level from the DM level. The neutral detergent fiber (NDF) levels were analyzed according to Van Soest and Robertson (1979) and the acid detergent fiber (ADF) levels were analyzed according to Goering and Van Soest (1970) by ANKOM® fiber analyzer.

The in vitro dry matter digestibilities (IVDMD) of the samples were determined according to the method of Tilley and Terry (1963) modified by Marten and Barnes (1979). For in vitro digestibility, rumen fluid was obtained from a previously cannulated Holstein cow. The cow was fed with alfalfa hay at a level of 1.5 times the maintenance level during the 10 days before the start of rumen fluid collection. Rumen fluid collected was filtered through a 4-layer cheesecloth before using as an inoculant to detect IVDMD. For metabolizable energy (ME, Mcal/ kg) and net energy (NE) for lactation (NE_L, Mcal/kg) values;

ME, (Mcal/kg) = Digestible energy × 0.82

NE_L, (Mcal/kg) = 0.00245 × Total Digestible Nutrition (TDN) - 0.12.

were calculated with equations.

Furthermore, Flieg points were calculated from the dry matter and pH values of silages using the following formula (Kurtoğlu, 2011);

Flieg Points = 220 + (2 x % Dry matter - 15)-(40 x pH)

According to this, silage quality evaluation was made with the scores obtained as 0-20 poor, 21-40 intermediate, 41-60 satisfactory, 61-80 good, and 81-100 very good.

All data of the study were subjected to analysis of variance by using the general linear model (GLM) procedure by the SAS (1998) program. The effects of different mixing ratios of silages and additives, the interactions between mixing ratios and additives were also determined. The differences among the groups were evaluated with Tukey's multiple range tests in the statistical significance level of P<0.05.

RESULTS

The pH, organic acid and ammonia-N values of the mixed silages are given in Table 1. According to this, the pH value of the mixture containing 60% feed peas was the highest at 4.45±0.07, and a significant difference (P<0,05) was found between the silage containing 80% FP with a pH value of 4.24±0.08. It was determined that LA, one of the organic acids, was significantly higher (P<0,05) in the mixed silage containing 80% FP compared to silages containing 20% and 40% FP. The AA level was significantly higher (P<0,05) in the mixed silage containing 60% FP compared to the others. Propionic acid and butyric acid levels were similarly found in all mixed silages (P>0,05). The highest ammonia-N level in the mixed silages was obtained in the mixed silage containing 80% FP with 1.10±0.06% and, it was found significantly higher than the others(P<0,05). When the effect of the additives on the silages was examined, it was determined that the inoculant increased the LA level significantly compared to the control silages. The effect of molasses on LA was similar to both control silages and inoculant-added silages (P<0,05). It was determined that the additives had no effect on other organic acid levels, pH, and ammonia-N parameters (P>0,05). Mixture level × additive interaction was determined at pH, LA, and ammonia-N levels. The pH level was higher (P<0,05) with the effect of inoculant in the mixture silage containing 60% FP. Inoculant

Table 1: Fermentation parameters of silages

	pH	LA	AA	PA	BA	Ammonia-N
Mixture proportion						
20% FP 80% RG	4.27 ± 0.05^{ab}	2.40 ± 0.24^{b}	0.25 ± 0.02^{b}	0.01±0.00	0.00±0.00	0.84 ± 0.05^{b}
40% FP 60% RG	4.32 ± 0.03^{ab}	2.53 ± 0.14^{b}	0.25 ± 0.01^{b}	0.00±0.00	0.00±0.00	0.91 ± 0.04^{b}
60% FP 40% RG	4.45 ± 0.07^{a}	2.80 ± 0.15^{ab}	0.42 ± 0.06^{a}	0.00±0.00	0.01±0.00	0.95 ± 0.04^{b}
80% FP 20% RG	4.24 ± 0.08^{b}	3.04 ± 0.17^{a}	0.27 ± 0.02^{b}	0.00±0.00	0.02±0.01	1.10 ± 0.06^{a}
P value	**0.03**	**0.01**	**0.001**	**0.83**	**0.40**	**<0.001**
Additive						
Control	4.25±0.06	2.50 ± 0.21^{b}	0.34±0.05	0.00±0.00	0.01±0.01	0.99±0.07
Inoculant	4.36±0.06	2.92 ± 0.13^{a}	0.30±0.02	0.00±0.00	0.00±0.00	0.90±0.03
Molasses	4.35±0.04	2.67 ± 0.12^{ab}	0.25±0.02	0.00±0.00	0.01±0.00	0.95±0.04
P value	**0.18**	**0.04**	**0.06**	**0.37**	**0.56**	**0.15**
Mixture Level x Additive	**0.01**	**<0.001**	**0.26**	**0.10**	0.18	<0.001
20% FP 80% RG						
Control	4.17±0.06	1.40 ± 0.07^{b}	0.24±0.05	0.00±0.00	0.00±0.00	0.71 ± 0.04^{b}
Inoculant	4.21±0.08	2.84 ± 0.27^{a}	0.29±0.02	0.01±0.00	0.00±0.00	0.88 ± 0.07^{ab}
Molasses	4.43±0.04	2.97 ± 0.16^{a}	0.21±0.03	0.01±0.00	0.01±0.01	0.93 ± 0.09^{a}
P value	**0.08**	**<0.001**	**0.60**	**0.20**	**0.79**	**0.05**
40% FP 60% RG						
Control	4.36±0.01	2.66±0.20	0.27±0.01	0.00±0.00	0.00±0.00	0.88±0.07
Inoculant	4.35±0.02	2.68±0.28	0.24±0.01	0.01±0.00	0.00±0.00	0.90±0.05
Molasses	4.24±0.08	2.26±0.25	0.24±0.02	0.00±0.00	0.00±0.00	0.95±0.08
P value	**0.6**	**0.33**	**0.91**	**0.56**	0.99	0.75
60% FP 40% RG						
Control	4.31 ± 0.09^{b}	2.41 ± 0.11^{b}	0.57 ± 0.16^{a}	0.00±0.00	0.00±0.00	1.02±0.03
Inoculant	4.70 ± 0.07^{a}	3.28 ± 0.18^{a}	0.37 ± 0.05^{b}	0.01±0.00	0.01±0.00	0.93±0.08
Molasses	4.35 ± 0.08^{b}	2.72 ± 0.27^{ab}	0.32 ± 0.03^{b}	0.00±0.00	0.01±0.01	0.90±0.09
P value	**0.01**	**0.03**	**0.01**	**0.11**	**0.69**	**0.39**
80% FP 20% RG						
Control	4.18±0.21	3.52 ± 0.30^{a}	0.29±0.03	0.01±0.00	0.04 ± 0.04^{a}	1.37 ± 0.06^{a}
Inoculant	4.16±0.05	2.88 ± 0.28^{b}	0.31±0.02	0.00±0.00	0.00 ± 0.00^{b}	0.91 ± 0.03^{b}
Molasses	4.39±0.08	2.71 ± 0.18^{b}	0.21±0.03	0.00±0.00	0.00 ± 0.00^{b}	1.02 ± 0.04^{b}
P value	**0.13**	**0.04**	**0.41**	**0.11**	0.02	<0.001

FP: Fodder pea, RG: Rye grass, LA: Lactic acid, AA: Acetic acid,PA: Propionic acid, BA: Butyricacid and molasses increased (P<0,05) the LA level in the mixture silages containing 20% FP, while inoculant and molasses decreased (P<0,05) the LA level in the mixture silages containing 80% FP. While ammonia-N level increased (P<0,05) with molasses in silages containing 20% FP, it decreased (P<0,05) with both molasses and inoculant in those containing 80% FP.

The in vitro organic matter digestibility (IVDOM), ME, and NE_L levels of the mixed silages are shown in Table 2. The IVDOM, ME and NE_L levels of the mixed silages were highest in silages containing 80% FP with 72.04±1.74, 3.18±0.08 and 1.65±0.04, respectively. The IVDOM, ME, and NE_L levels of the mixed silages were lowest in 60% FP mixtures with 64.30±1.29, 2.84±0.06, 1.46±0.03, respectively. These values differed significantly from silages containing 20%and 40% FP (P<0.05). Besides, the effect of inoculant and molasses additives on IVDOM, ME and NE_L levels was significant; the effect of molasses was higher than inoculant (P<0.05). Mixture level × additive interaction occurred in all three parameters, and it was determined that the effect of the inoculant increased in silages containing 80% FP.

The nutrient content levels of the obtained silages are given in Table 3. Accordingly, the highest dry matter (DM) and ash levels were 36.78±0.65 and 7.12±0.26 in the mixed silage containing 40%FP, respectively. The lowest DM and ash levels were 33.83±0.74 and 5.63±0.28, in the mixed silage containing 60% FP, respectively. The difference between them was

Table 2: Digestibility and energy values of silages

	IVDOM, %OM	ME, (Mcal/kg)	NE_L, (Mcal/kg)
Mixture proportion			
20% FP 80% RG	68.99±0.81[b]	3.04±0.05[b]	1.57±0.02[b]
40% FP 60% RG	68.45±1.39[b]	3.02±0.06[b]	1.56±0.03[b]
60% FP 40% RG	64.30±1.29[c]	2.84±0.06[c]	1.46±0.03[c]
80% FP 20% RG	72.04±1.74[a]	3.18±0.08[a]	1.65±0.04[a]
P value	**<0.001**	**<0.001**	**<0.001**
Additive			
Control	64.92±0.65[c]	2.86±0.03[c]	1.47±0.02[c]
Inoculant	66.98±1.24[b]	2.95±0.05[b]	1.52±0.03[b]
Molasses	73.44±0.96[a]	3.24±0.04[a]	1.68±0.02[a]
P value	**<0.001**	**<0.001**	**<0.001**
Mixture Level x Additive	**<0.001**	**0.001**	**0.001**
20% FP 80% RG			
Control	66.49±0.98[b]	2.93±0.04[b]	1.51±0.02[b]
Inoculant	68.58±0.32[b]	3.02±0.01[b]	1.56±0.01[b]
Molasses	71.90±1.11[a]	3.17±0.05[a]	1.64±0.03[a]
P value	**0.006**	**0.006**	**0.006**
40% FP 60% RG			
Control	66.42±0.36[b]	2.92±0.01[b]	1.51±0.01[b]
Inoculant	64.36±0.64[b]	2.84±0.03[b]	1.46±0.02[b]
Molasses	74.58±1.07[a]	3.29±0.05[a]	1.71±0.03[a]
P value	**<0.001**	**<0.001**	**<0.001**
60% FP 40% RG			
Control	61.39±1.17[b]	2.71±0.05[b]	1.38±0.03[b]
Inoculant	61.64±0.74[b]	2.72±0.03[b]	1.39±0.02[b]
Molasses	69.87±0.96[a]	3.08±0.04[a]	1.59±0.02[a]
P value	**<0.001**	**<0.001**	**<0.001**
80% FP 20% RG			
Control	65.37±0.44[c]	2.88±0.02[c]	1.48±0.01[c]
Inoculant	73.32±1.88[b]	3.23±0.08[b]	1.68±0.05[b]
Molasses	77.43±2.10[a]	3.41±0.09[a]	1.78±0.05[a]
P value	**<0.001**	**<0.001**	**<0.001**

FP: Fodder pea, RG: Rye grass, IVDOM: In vitro digestibility of organic matter, OM: Organic matter, ME: Metabolic energy, NE_L: Net energy for lactation statistically significant (P<0.05) and they were found to be similar to the others (P>0.05). Organic matter (OM) levels were highest with 94.21±0.22 in silages containing 60% FP, and lowest with 92.88±0.26 in silages containing 40% FP. While the difference between these is significant (P<0, 05), it was found to be similar to the others (P>0.05). NDF, ADF, and HP levels of silages were similarly low in those containing 20% and 40% FP and higher in those containing 60% and 80% of FP. While the low- and high-level silages were statistically similar in themselves, it was determined that there was a difference between them (P<0.05). Only molasses was found to be effective on DM and ADF levels. While it increased the DM level significantly, it decreased the ADF level significantly (P<0.05). It was determined that the inoculant had no effect. Mixture level × additive interaction occurred only at the NDF level, and it was observed that it decreased significantly with the effect of molasses in silages containing 40% FP (P<0.05).

The physical properties and Flieg scores of the mixed silages are given in Table 4. Accordingly, different ratio mixtures of FP and RG had no effect on odor and color (P>0.05). It was determined that the structure of silages with an 80% FP ratio was better than the other mixture ratios (P<0.05). The Flieg score was significantly lower in silages containing 60% FP compared to silages containing 20% and 40% FP(P<0.05). While both additives were ineffective on physical properties (P>0.05), Flieg score increased by molasses (P<0.05). The mixture level × additive in-

Table 3: Nutrient contents of silages

	DM	ASH	OM	CP	NDF	ADF
Mixture proportion						
20% FP 80% RG	36.19±0.91[ab]	6.23±0.26[ab]	93.77±0.26[ab]	10.87±0.16[b]	42.04±1.47[b]	25.86±0.71[b]
40% FP 60% RG	36.78±0.65[a]	7.12±0.26[a]	92.88±0.26[b]	11.75±0.16[b]	41.38±1.37[b]	26.27±1.18[b]
60% FP 40% RG	33.83±0.74[b]	5.63±0.28[b]	94.21±0.22[a]	13.09±0.30[a]	46.48±0.68[a]	30.68±0.76[a]
80% FP 20% RG	35.16±0.89[ab]	6.39±0.34[ab]	93.61±0.34[ab]	13.58±0.42[a]	46.95±1.01[a]	30.26±0.75[a]
P value	**0.02**	**<0.01**	**0.02**	**<0.001**	**<0.001**	**<0.001**
Additive						
Control	33.86±0.66[b]	6.09±0.27	93.78±0.22	12.10±0.33	44.78±1.06	28.84±0.95[a]
Inoculant	34.90±0.48[b]	6.40±0.33	93.60±0.33	12.54±0.42	45.08±1.07	29.63±0.76[a]
Molasses	37.72±0.69[a]	6.54±0.22	93.46±0.22	12.47±0.36	42.78±1.35	26.35±0.88[b]
P value	**<0.001**	**0.44**	**0.65**	**0.40**	**0.15**	**0.002**
Mixture Level x Additive	**0.84**	**0.43**	**0.55**	**0.56**	**0.01**	**0.06**
20% FP 80% RG						
Control	34.02±2.19[b]	5.99±0.36	94.01±0.36	10.68±0.16	40.77±1.35	25.16±1.03
Inoculant	36.25±1.07[ab]	6.27±0.41	93.73±0.41	11.05±0.45	40.83±2.60	26.28±0.82
Molasses	38.31±0.39[a]	6.45±0.66	93.55±0.66	10.87±0.13	44.51±3.49	26.23±1.88
P value	**<0.05**	**0.81**	**0.80**	**0.87**	**0.24**	**0.77**
40% FP 60% RG						
Control	36.23±0.59[ab]	7.23±0.42	92.77±0.42	12.08±0.29	42.61±1.56[a]	27.20±0.84[a]
Inoculant	35.02±0.77[b]	7.02±0.68	92.98±0.68	11.34±0.32	45.27±1.63[a]	29.75±1.69[a]
Molasses	39.10±0.85[a]	7.12±0.26	92.89±0.26	11.83±0.12	36.25±1.15[b]	21.86±0.96[b]
P value	**0.053**	**0.96**	**0.96**	**0.56**	**0.003**	**<0.001**
60% FP 40% RG						
Control	32.11±0.45	4.94±0.40	94.56±0.15	12.76±0.24	46.86±1.88	31.87±2.09
Inoculant	33.29±1.10	5.39±0.33	94.61±0.33	13.60±0.83	45.70±0.44	30.79±0.82
Molasses	36.08±1.35	6.56±0.35	93.44±0.35	13.51±0.28	46.88±1.02	29.38±0.44
P value	**0.06**	**0.08**	**0.18**	**0.42**	**0.86**	**0.37**
80% FP 20% RG						
Control	33.05±0.58[b]	6.22±0.32	93.78±0.32	12.87±0.97	48.87±0.90	31.13±1.26
Inoculant	35.03±0.45[ab]	6.91±0.95	93.09±0.95	14.18±0.13	48.51±1.77	31.69±1.27
Molasses	37.41±2.24[a]	6.04±0.36	93.61±0.34	13.68±0.81	43.46±1.05	27.95±0.50
P value	**0.04**	**0.44**	**0.42**	0.18	0.06	0.09

FP: Fodder pea, RG: Rye grass, DM: Dry matter, OM: Organic matter, CP: Crude protein,NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Table 4: Physically properties and Flieg points of silages

	Odor (point)	Structure (point)	Color (point)	Total (point)	Flieg (point)
Mixture proportion					
20% FP 80% RG	10.00±0.95	2.75±0.28[b]	1.42±0.15	14.17±1.21	106.62±1.89[a]
40% FP 60% RG	12.17±0.87	3.08±0.29[b]	1.67±014	16.92±1.13	106.84±2.28[a]
60% FP 40% RG	10.00±1.28	3.00±0.30[b]	1.67±0.14	14.67±1.62	94.55±2.94[b]
80% FP 20% RG	9.67±1.49	4.00±0.00[a]	1.92±0.08	15.58±1.54	102.30±2.93[ab]
P value	**0.48**	**0.003**	**0.10**	**0.56**	**<0.001**
Additive					
Control	11.00±1.03	3.31±0.24	1.69±0.12	16.00±1.21	100.82±2.07[b]
Inoculant	10.00±1.00	2.88±0.26	1.69±0.12	14.56±1.18	100.57±3.09[b]
Molasses	10.37±1.07	3.44±0.22	1.62±0.13	15.44±1.25	106.35±2.01[a]
P value	**0.80**	**0.12**	**0.91**	**0.72**	**0.04**
Mixture Level x Additive	**0.85**	**0.08**	**0.55**	**0.69**	**0.002**
20% FP 80% RG					
Control	10.50±2.36	2.50±5.00	1.50±0.29	14.50±2.90	106.45±3.90
Inoculant	9.50±2.06	3.00±0.58	1.50±0.29	14.00±2.74	109.10±4.28
Molasses	10.00±0.00	2.75±0.48	1.25±0.25	14.00±0.41	104.31±1.43
P value	**0.95**	**0.67**	**0.69**	**0.99**	**0.64**
40% FP 60% RG					
Control	12.00±0.82	3.25±0.48[a]	1.50±0.29	16.75±1.11	105.98±2.32
Inoculant	10.50±2.36	2.00±0.00[b]	1.50±0.29	14.00±2.61	101.03±2.01
Molasses	14.00±0.00	4.00±0.00[a]	2.00±0.00	20.00±0.00	113.50±4.66
P value	**0.52**	**0.004**	**0.24**	**0.25**	**0.06**
60% FP 40% RG					
Control	10.50±2.36	3.50±5.00	1.75±0.25	15.75±3.10	96.82±2.68[a]
Inoculant	9.50±2.06	2.50±5.00	1.75±0.25	13.75±2.46	83.48±4.38[b]
Molasses	10.00±2.83	3.00±0.58	1.50±0.29	14.50±3.57	103.36±0.52[a]
P value	**0.95**	**0.22**	**0.69**	**0.85**	**0.001**
80% FP 20% RG					
Control	11.00±3.00	4.00±0.00	2.00±0.00	17.00±3.00	94.01±4.16[b]
Inoculant	10.50±2.36	4.00±0.00	2.00±0.00	16.50±2.36	108.65±2.38[a]
Molasses	7.50±2.75	4.00±0.00	1.75±0.25	13.25±2.93	104.22±5.82[a]
P value	**0.47**	**1.00**	**0.69**	**0.52**	**0.02**

FP: Fodder pea, RG: Ryegrass

teraction occurred only in the Flieg score. While Flieg score decreased significantly with inoculant in silages with 60% FP($P<0.05$), it increased with both inoculant and molasses in silages with 80% FP($P<0.05$).

DISCUSSION

The type of bacteria desired to be present in a quality silage is lactic acid-producing bacteria.For this, a sufficient level of easily soluble carbohydrates, an anaerobic environment, and a low pH (3.8-4.2) are required in the silage environment (Ergün et al., 2013). When Table 1 showing the fermentation parameters of silages is examined, the pH values of silages showed a quadratic change with decreasing RG level and increasing FP level. As FP level increased from 20% to 60%, pH values tended to increase in line with the findings of Seydoşoğlu (2019a). Seydoşoğlu expressed that this situation is due to decreasing fermentable carbohydrates, high protein content, and the increase in the release of ammonia by the breakdown of proteins. The numerical increase in ammonia-N levels also confirms this in the present study. While the pH value was expected to increase due to the increase in the ammonia-N level in the silage group containing 80% FP, it had the lowest pH.The growth of undesirable bacteria, yeast, and molds leads to decreased quality by increasing the pH of the silage (Aykan and Saruhan, 2018).Even though this situation is not visible in silages, when the structures of silages are examined in Table 4, it is seen that the structure of silages containing 20, 40, and 60% FP is lower than the others containing 80% FP.A high LA level is an indicator of quality in silage, and the LA level should be above 2%. Acetic acid can be found in some amounts (0.3-0.8%). Bacteria producing butyric acid are undesirable in silages as they break down proteins and cause amine and ammonia release(Kiraz and Kutlu, 2016; Uygur, 2019). In the present study, LA levels were above the specified limit and were determined in the group containing the highest 80% FP compatible with pH.Acetic acid level was found between the values stated in the group containing 60% FP, while the others were lower. Butyric acid levels of silages were almost non-existent. In the present study, it was determined that while the additives had an effect on the lactic acid levels of the silages, the inoculant additive was more effective. We determined in our previous similar study that the inoculant was more effective than the control groupin Hungarian vetch and rye grass silages (Şen et. al., 2022).

In the present study, both IVDOM and energy values were highest in the silage group containing 80% FP. This result supports the results of pH and LA levels of silages containing 80% FP in Table 1 in terms of quality. When the nutrient contents of the silages shown in Table 3 are examined, the high NDF and ADF levels of the silages containing 60% and 80% FP draw attention. While the low IVDOM and energy values occurring in silages containing 60% FP may be due to this, the adverse effect was observed in silages containing 80% FP.In addition to being higher in molasses than silage additives, both had a positive effect on digestibility and energy values. The positive effect of molasses on digestibility is consistent with the results of similar studies (Garipoğlu 2020; Gürsoy et al. 2023).It was stated by Zhang et al (2019) that molasses contains high dry matter and low fiber content. Therefore, there is a high amount of non-structural carbohydrates in the environment. Thus, it is expected to provide better fermentation. This status also supports the increase in IVDOM level of the molasses added group. Inoculant supplement also increased the IVDOM and energy values. However, in our previous study with rye grass and Hungarian vetch, it was determined that the inoculant additive did not affect the digestive and energy values (Şen et al., 2022).The difference between the presented study and our previous study is that feed peas are used instead of Hungarian vetch with rye grass in the silage mixture. However, Kara et al. (2021) determined that the difference between the digestibility of forage peas and Hungarian vetch silages was not significant, but it was higher in fodder peas with only a numerical difference.

The nutrient contents of silages are given in Table 3.Accordingly, dry matter and ash levels were similarly the highest in silages containing 40% FP, and the lowest in silages containing 60% FP. However, the dry matter levels of all groups were in the range of 30-40%, which are the expected levels in ideal silage material (Ergün et al., 2013). In addition, molasses additive increased the dry matter level as similar studies (Zhao et al., 2019; Garipoğlu et al., 2020).According to Zhao et al. (2019), the dry matter level increased with the addition of molasses was due to highdry matterin the structure of molasses.Crude protein ratios of silages increased with graminae ratios decreasing and legume ratios increasing in experiment groups.In similar studies (Geren, 2014; Gelir and Denli,2018; Gümüştaş and Turan, 2022), it was determined that the protein ratio increased as the amount of legume increased. Seydoşoğlu (2019b) also determined that as the ratio of legumes in the mixture increased, the protein ratio of silage increased as well, and stated that this was due to the high amount of protein in the structure of legumes. According to Turan (2019), the increased HP value due to the increase in the legume ratio of the mixture decreases the ADF and NDF values. However, in the present study, NDF and ADF levels were higher in silages containing 60% and 80% FP than in other groups. Öten et al. (2016) reported that the NDF ratio decreased with increasing alfalfa ratio in corn-alfalfa mixed silages, while the ADF ratio did not change. Arslan et al. (2016) determined that while NDF level did not change with increasing soybean in corn-soybean mixed silages, ADF level increased. Different plant species used, harvest time, silage-making techniques and ecological conditions may be the reason for the different results obtained from similar studies (Seydoşoğlu, 2019a; Turan, 2020).In addition, it is seen that the NDF level decreased numerically with the molasses contribution, while the ADF level decreased significantly. The reasons for this decrease in NDF and ADF values may be the dilution effect due to the low NDF and ADF content of molasses, the increase of fermentation with molasses, and the hydrolysis effect of the acid environment (Chaji et al., 2020).

In addition to chemical analyses that are more costly and require laboratory conditions to determine silage quality, another analysis method that is less costly, easier and without laboratory conditions is the physical analysis method. Odor, color, and structure are criteria used to determine silage quality by physical analysis methods (Aykan and Saruhan, 2018). Physical properties and Flieg scores of silages are shown in Table 4.Accordingly, while the odor and color scores of the silage groups were similar, the silage group containing 80% FP in structure scores was significantly higher than the other groups.The most important factor in the preservation of the structure is to ensure a successful fermentation.In this way, mold formation and deterioration do not occur with increasing lactic acid concentration in a short time, and the structure of plant leaves and stalks can be preserved (Gümüştaş and Turan, 2022).When the total scores of the groups are examined in the present study, it is determined that there is no significant difference between the groups. When the quality evaluation is made, it is seen that all groups are at a "good" level. In similar studies (Seydoşoğlu 2019b; Gümüştaşand Turan, 2022), it was determined that the total score decreased as the ratio of grasses in silage increased and the ratio of legumes decreased. This situation was evaluated by Seydoşoğlu (2019b) as the expected situation due to the high amount of easily fermentable carbohydrates in the structure of the graminae species and low amount in the legume species. In the results of Gümüştaş and Turan (2022), it is seen that the quality status of legumes and grasses mixed silages changed from "very good" to "good" as the legume ratio increased and decreased to "intermediate" at the 100% legume level. It was determined that additives did not have significant effects on the physical properties of silages. The Flieg score,which evaluates silage quality by using dry matter and pH values of silages of the group containing 60% FP from the mixed silages, was the lowest. However, this result obtained was also in the "very good" range. It was stated by Aykan and Saruhan (2018) that Flieg scores increased in parallel with the increase in the proportion of grasses in the mixture. This increase was probably due to decreased pH with increasing amount of soluble carbohydrates. The results in the present study were not similar. When dry matter levels are examined in Table 3, it is seen that there is a similar change to the Flieg score. Since the highest pH value was in this group, the Flieg score was lower than the others. This situation was also determined under the influence of additives and the highest Flieg score was in the molasses-added group. This is due to the easily fermentable carbohydrates contained in molasses (Demirci et al., 2023).

CONCLUSION

As a result, although mix silages containing 80% FP had high ammonia-N, excellent fermentation was observed with low pH value and high LA level. It has been determined that the addition of inoculant can contribute positively to the fermentation of silages. Although NDF and ADF levels increased, mixed silages with FP at 80% levels have higher in vitro digestibility and energy levels. Each of the additives positively affected the in vitro digestibility of silages. While the dry matter levels of silages can be increased with the addition of molasses,the ADF levels can be reduced. Also, the quality of silages containing FP up to 80% can be increased with the addition of molasses.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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