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The effect of microbial inoculants and molasses on quality and in vitro digestibility of silages prepared with different proportions of ryegrass and Hungarian vetch

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Abstract: In this study, it was aimed to determine the effect of microbial inoculants and molasses on the quality and in vitro digestibility of the silages ensiled from the cultivation of different proportions of ryegrass-Hungarian vetch mixtures grown in Central Anatolian conditions. For this purpose, 5% molasses and 10 g/ton (1.25×10^{11} CFU/g) inoculant were added to silages of ryegrass (*Lolium multiflorum L.*) containing 20%, 40%, 60%, and 80% Hungarian vetch (*Vicia pannonica L.*), under laboratory conditions. The prepared silages were opened at the end of 60 days and their physical analysis, chemical contents, fermentation parameters, in vitro digestibilities using rumen inocula obtained from cannulated Holstein cow, and energy levels were determined. In the study, it was determined that the microbial inoculant increased the lactic acid level significantly ($p < 0.05$). In vitro digestibility of organic matter and energy values increased in silages containing 60% HV compared to other silages and with both additives ($p < 0.05$). The neutral detergent fiber levels increased in mixtures containing 40% and 60% Hungarian vetch ($p < 0.05$). The increase in the vetch ratio affected the external appearance of the silages negatively ($p < 0.05$), and the physical properties were adversely affected by the inoculant ($p < 0.05$). As a result, it was determined that Hungarian vetch can be mixed with ryegrass up to 80% with and without inoculant and molasses additive to obtain high-quality silage. But the highest digestibility values were obtained when Hungarian vetch was mixed at a 60% level. It would be more appropriate to prefer molasses to avoid undesired changes in physical properties.

Keywords: Ryegrass, Hungarian vetch, silage, microbial inoculant, molasses, in vitro digestibility

1. Introduction

Roughage is of great importance in cattle and sheep breeding. Using the affordable roughage instead of the more expensive concentrate feed provides the opportunity to make economical farming in the enterprises [1]. Feed expenses constitute approximately 70% of the total expenses in an enterprise. Among these expenses, while the rate of roughage is 78%, concentrated feed constitutes 22%. Roughage sources consist of meadows and pastures, forage crops, and stalks [2]. It is observed that while the number of animals has increased by about 80% in the last ten years in Turkey, meadow and pasture areas have remained the same[1]. Despite the increasing number of animals, the fact that the meadow and pasture areas remain the same means a decrease in the grazing area per unit animal. In addition, due to early, unplanned and excessive grazing in these areas, erosion of vegetation has also occurred [3]. Stalks and straws, on the other hand, are bulky stuff and provide only mechanical satisfaction for animals, not much nutrient [4]. The roughage production level in agricultural lands is very low and the quality is also low. Cultivated gramineous varieties are utilized as silage, legumes are mostly used as fresh or hay and very less of it ensilaged [5]. Green fodders, which are a source of high-quality roughage in beef and dairy cattle nutrition, are available only at certain times of the year. At other times of the year when quality roughage is not available, producers use cereal straws with low nutritional value. This situation causes low yields even in high-yielding breeds. When high-quality roughage is not available, silage forages rich in water can meet animals' needs [6].

Gramineous forage plants provide a desirable fermentation due to the high level of easy soluble carbohydrates in their structure and therefore their silages are high quality [7]. However, due to the low protein content of silages made from cereal grains, one way of improving the protein content of such silages is to plant cereal grains together with legume forage plants at different rates. In

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[1] TÜİK, Turkish Statistical Institute (2021). Website https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1, [accessed: 09.03.2021].

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this way, protein levels of energy-rich grain silages can be increased with legumes [8]. On the other hand, when legume forages like vetch plant is made in silages, it is very difficult to obtain high-quality silage due to a high buffer capacity. For this reason, silage quality can be increased by adding cereal plants with high carbohydrate content. The species of vetch to be used and the suitable ratio for the climatic conditions of the region where it is planted should be determined. Planting mixed with grains of vetches is also effective in preventing rot by clinging to grains with shoots [7].

To improve the carbohydrate content, additives with high sugar content such as cereal grains, molasses, and grape pomace are also used [9]. To improve silage quality, bacterial inoculants to provide high fermentation along with carbohydrate additives are also used [10]. Among the vetch species, Hungarian vetch (*Vicia pannonica L.*) is a durable plant that is resistant to drought and harsh climatic conditions and does not need high levels of water [11].

The aim of this study was to determine the effects of molasses and microbial inoculant addition into silages obtained from the mixture of ryegrass and Hungarian vetch at different ratios grown in Central Anatolian conditions without irrigation and artificial fertilizers in arid climate conditions.

2. Materials and methods

The study was carried out in Kırıkkale University Campus experimental area in Kırıkkale province with continental climate in the Central Anatolian region of Turkey (39°53'04.9" N, 33°26'20.0" E). The annual average rainfall of Kırıkkale province is 405 mm. In the period of the experiment, the total precipitation in the region was 314.3 mm, below the annual average. The summers of the region are hot and dry, and the winters are cold and rainy. The soil organic matter level of the study area is low (1.33%), moderately calcareous (12.15%), slightly alkaline (pH = 7.73), salt-free (0.10 EC (dS/m)), and sufficient level of potassium (216 ppm). The soil nitrogen (0.18%) and phosphorus (3.13 ppm) levels are also low. Ryegrass (RG) (*Lolium multiflorum L.*) and Hungarian vetch (HV) (*Vicia pannonica L.*) species were planted in 5 × 1.5 m^2 plots as 3 replications. Each plot was planted as 5 rows of RG and 5 rows of HV. The seeds were sown on the rows with 15 cm distance between. The amounts of the seeds sowed were 6 kg/da for RG and 10 kg/da for HV. Mixing ratios were determined as 20%, 40%, 60%, 80% and the seeds were sown at these rates for each plot. Irrigation and fertilization were not applied to the plots. Harvesting was done manually from 1 m^2 area of each plot. After harvest, approximately 10 kg of fresh material from each mixing ratio was chopped into 2–3 cm lengths. The prepared material was laid on an area of approximately 2 m^2. Molasses and microbial inoculants were applied and homogeneously mixed. Each of the mixtures containing 4 different ratios of HV was compacted by hand into a total of 48 jars of 1.5 L, including control, 5% molasses, and 10 g/t inoculant (1.25 × 1011 CFU/g) groups in 4 replications. Inoculant used in the study was obtained from DU PONT 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 ensiling, the jar lids were tightly closed and stored in a room at 20–25 °C for 60 days. At the end of 60 days, all silages were opened and their physical analysis, chemical contents, fermentation parameters, in vitro digestibility, and energy levels were determined.

Silage samples were opened and scored by three experts in terms of odor, color, and structure according to DLG [12]. Then, 100 mL of distilled water was added to the 25 g wet silage sample and mixed thoroughly with a mixer, and the liquid part was filtered. The pH of the filtrate was measured with a digital pH meter (HANNA, HI 2221) [13]. Some of the filtrates was stored at –20 °C until the organic acid analysis. The ammonia-N concentrations of the silages were determined by the Kjeldahl distillation method using the same filtrate [14].

The lactic acid (LA) concentration in the filtrate was determined according to a modified spectrophotometric method [15] by Barnett [16]. Analysis of other organic acids (butyric acid (BA), propionic acid (PA), and acetic acid (AA)) was also determined according to a modified spectrophotometric method by Tekin and Kara [15]. To determine the dry matter of silage samples, silage samples were first predried with air drying, then, subsamples were dried at 65 °C for 72 h. Dried samples were ground in a grinder mill to a particle size of 1 mm for other chemical analysis and the results were expressed as dry matter. The crude protein (CP), ash levels were determined according to the method reported by the AOAC [17]. The organic matter (OM) level was calculated as the remaining amount by subtracting the ash level from the DM level. The neutral detergent fiber (NDF) levels were analyzed according to Van Soest and Robertson [18] and the acid detergent fiber (ADF) levels were analyzed according to Goering and Van Soest [19] by using ANKOM® fiber analyzer.

The in vitro dry matter digestibilities (IVDMD) of the samples were determined according to the method of Tilley and Terry [20] modified by Marten and Barnes [21]. Holstein cow with rumen cannula was fed with alfalfa hay at a 1.5 maintenance level starting 10 days before fluid collection. Rumen fluid collected from Holstein cow with rumen cannula was used as an inoculant to detect IVDMD after filtering through a 4-layer cheesecloth. Metabolizable energy (ME, Mcal/kg) and lactation net energy values (NE_L, Mcal/kg) of the samples were calculated using the following formulas [22]:

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

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

The data obtained from the study were subjected to analysis of variance with the General Linear Model using the SAS program. The effects of different mixing ratios and silage additives, the interactions of mixing ratios and silage additives were also determined. The differences between the experimental groups were expressed with Tukey's multiple range tests considering the statistical significance level of $p < 0.05$.

3. Results

The organic acid contents and pH parameters of Ryegrass and Hungarian vetch mixture silages are given in Table 1. Accordingly, the difference between the LA and AA levels of the mixtures at different ratios is statistically significant ($p < 0.05$). It was observed that the LA level was significantly higher in the group with 60% HV than in the groups with 20% and 80% levels. The AA level was significantly higher in the 60% mixture than in the 40% mixture. The effect of the additive was only on the amount of LA, and it was observed that there was a significant increase in the inoculant groups compared to the control and molasses groups ($p < 0.05$). Mixture level × additive interaction was observed at LA, AA, and ammonia nitrogen levels ($p < 0.05$).

In vitro digestibility of organic matter (IVDOM) and energy values of the mixed silages are given in Table 2. Both IVDOM and energy values of the mixture containing only 60% HV were found significantly higher than the others ($p < 0.05$). The effects of additives on IVDOM and energy values of the mixtures were positive by both inoculant and molasses ($p < 0.05$). There was a mixture level × additive interaction on IVDOM and energy values ($p < 0.05$).

The nutrient contents of the mixtures are shown in Table 3. There was a difference in the DM of the mixture ratios, and the mixture containing 40% HV was similar to the control group, while the DM of the groups containing 60% and 80% HV was lower ($p < 0.05$). Amounts of NDF increased significantly at 40% and 60% rates compared to 20% and 80% rates of no additive silages ($p < 0.05$). It is seen that the additives do not have a significant effect on the mixtures ($p > 0.05$). The CP levels of the 40% HV mixture have mixture level × inoculant interaction ($p < 0.05$). There was also an interaction between the mixture level × additive for the NDF and ADF levels of the mixtures ($p < 0.05$).

As the quality criteria of silages, their physical and sensory (smell, structure, color) properties and their Flieg scores are given in Table 4. It was determined that there was a significant difference between the mixing ratios only in the mixtures at 20% and 80% levels ($p < 0.05$). When the effects of the additives on the mixtures are examined, it was seen that the inoculant has a negative effect on all parameters except the Flieg score ($p < 0.05$). There was a mixing ratio × additive interaction in terms of external appearance and physical structure ($p < 0.05$).

4. Discussion

To obtain quality silage, feed materials with high carbohydrate and low-protein contents are preferred. This results in the formation of high-energy but low-protein feed material. However, a good feed should have a balanced energy and protein content. For this purpose, energy-rich feed materials can be mixed with protein-rich feed materials or mixed planting. The pH of the high-quality silage to be formed by a good fermentation should be acidic and the lactic acid level should be high.

In the current study, while HV was expected to increase the pH due to its high protein content according to Seydoşoğlu [23] and Turan [11], the addition of up to 80% did not increase the pH. This may be related to the fact that the protein level of ryegrass (13%– 16%) is not as low as cereals [24] and therefore does not have a significant effect on the pH of the silage medium, although its amount has decreased. The ammonia-N levels of the groups were also similar. When HV is mixed at the level of 60%, while the highest lactic acid level is obtained, the acetic acid level has also increased. The effect of this on the pH level was seen numerically. The amount of lactic acid increased significantly with the addition of inoculant. It has been reported by Turan [11] that it is recommended to use inoculant containing lactic acid bacteria, primarily *Lactobacillus plantarum* with homofermentative properties, to increase the level of lactic acid in mixed grass and legume silages. However, the amount of lactic acid decreased in the mixture containing 80% HV despite the addition of inoculant. This may be due to the increase in the silage buffer capacity and the insufficient effect of the inoculant [9]. The fact that the amount of lactic acid is higher than acetic acid with the effect of inoculant additive is compatible with the study of the İnan Erbil [25]. İnan Erbil [25] stated that this may be due to the dominance of lactic acid bacteria with the contribution of inoculants and the inactivity of other acid-producing bacteria.

In the current study, it was determined that molasses and inoculant additives increase the digestion of silages. Bingöl et al. [26] reported that the addition of 5% molasses

Table 1. Fermentation parameters of silages, DM%.

	pH	LA	AA	PA	BA	Ammonia-N
M Level						
80% RG+20% HV	4.44 ± 0.07	0.99 ± 0.12^{b}	0.21 ± 0.02^{ab}	0.00 ± 0.00	0.00 ± 0.00	0.91 ± 0.03
60% RG+40% HV	4.36 ± 0.06	1.21 ± 0.08^{ab}	0.17 ± 0.02^{b}	0.00 ± 0.00	0.00 ± 0.00	0.89 ± 0.04
40% RG+60% HV	4.53 ± 0.10	1.39 ± 0.20^{a}	0.24 ± 0.02^{a}	0.00 ± 0.00	0.00 ± 0.00	0.92 ± 0.04
20% RG+80% HV	4.28 ± 0.05	1.05 ± 0.07^{b}	0.23 ± 0.02^{ab}	0.00 ± 0.00	0.00 ± 0.00	0.92 ± 0.04
P-value	**0.10**	**<0.001**	**0.02**	**1.00**	**1.00**	**0.93**
Additive						
Control	4.46 ± 0.06	0.94 ± 0.08^{b}	0.22 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.90 ± 0.02
Inoculant	4.28 ± 0.05	1.56 ± 0.12^{a}	0.19 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.93 ± 0.04
Molasses	4.47 ± 0.08	0.99 ± 0.07^{b}	0.22 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.90 ± 0.04
P value	**0.06**	**<0.001**	**0.21**	**1.00**	**1.00**	**0.68**
HV Level × Additive	**0.50**	**<0.001**	**<0.001**	**1.00**	**1.00**	**0.002**
20% HV						
Control	4.41 ± 0.14	0.75 ± 0.13^{b}	0.20 ± 0.04^{b}	0.00 ± 0.00	0.00 ± 0.00	0.87 ± 0.01^{b}
Inoculant	4.41 ± 0.19	1.48 ± 0.03^{a}	0.14 ± 0.01^{b}	0.00 ± 0.00	0.00 ± 0.00	0.84 ± 0.01^{b}
Molasses	4.52 ± 0.06	0.75 ± 0.12^{b}	0.30 ± 0.01^{a}	0.00 ± 0.00	0.00 ± 0.00	1.02 ± 0.07^{a}
P value	**0.77**	**<0.001**	**0.001**			**0.05**
40% HV						
Control	4.54 ± 0.12	1.05 ± 0.16^{b}	0.17 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.85 ± 0.03^{b}
Inoculant	4.25 ± 0.04	1.45 ± 0.13^{a}	0.17 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	1.06 ± 0.06^{a}
Molasses	4.29 ± 0.10	1.12 ± 0.03^{b}	0.16 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.77 ± 0.02^{b}
P value	**0.22**	**0.02**	**0.90**			**0.003**
60% HV						
Control	4.54 ± 0.11	0.66 ± 0.03^{c}	0.26 ± 0.04^{a}	0.00 ± 0.00	0.00 ± 0.00	0.99 ± 0.06
Inoculant	4.31 ± 0.02	2.25 ± 0.13^{a}	0.17 ± 0.02^{b}	0.00 ± 0.00	0.00 ± 0.00	0.84 ± 0.04
Molasses	4.74 ± 0.27	1.27 ± 0.12^{b}	0.30 ± 0.04^{a}	0.00 ± 0.00	0.00 ± 0.00	0.94 ± 0.08
P value	**0.06**	**<0.001**	**<0.01**			**0.16**
80% HV						
Control	4.36 ± 0.10	1.29 ± 0.09^{a}	0.26 ± 0.02^{a}	0.00 ± 0.00	0.00 ± 0.00	0.91 ± 0.04
Inoculant	4.14 ± 0.03	1.06 ± 0.07^{ab}	0.29 ± 0.01^{a}	0.00 ± 0.00	0.00 ± 0.00	0.99 ± 0.10
Molasses	4.33 ± 0.06	0.80 ± 0.02^{b}	0.14 ± 0.01^{b}	0.00 ± 0.00	0.00 ± 0.00	0.86 ± 0.05
P value	**0.40**	**<0.01**	**0.002**	**1.00**	**1.00**	**0.25**

LA: Lactic acid. AA: Acetic acid. PA: Propionic acid. BA: Butyric acid

to the silage of the sainfoin obtained from two different harvest periods increased the digestibility. Bingöl et al. [27] observed that the addition of 2%, 4%, 6% molasses to the silages prepared from barley and sainfoin mixtures obtained from two different harvest periods, increased the digestibility of the silages of both harvest periods at all mixing ratios. As a result of these studies, it was stated that the effect of molasses on digestibility may be due to its positive effect on fermentation. Although they reported that NDF and ADF levels may decrease due to the fermentation developed with the effect of molasses additive, this was not observed in the current study. Desta et al. [28] also reported that NDF and ADF content in Napier grass silage decreased with molasses addition. They stated that the organic acids produced during the ensiling, or the direct acid addition could increase the hydrolysis of structural carbohydrates. On the other hand, Li et al. [29] determined that neither molasses nor inoculant additive

Table 2. In vitro digestibility of organic matter and energy values of silages.

	IVDOM, %OM	ME, (Mcal/kg)	NE$_L$, (Mcal/kg)
M Level			
80% RG+20% HV	64.22 ± 2.26^{b}	2.83 ± 0.10^{b}	1.46 ± 0.05^{b}
60% RG+40% HV	60.94 ± 1.61^{b}	2.69 ± 0.07^{b}	1.37 ± 0.04^{b}
40% RG+60% HV	68.48 ± 1.72^{a}	3.02 ± 0.08^{a}	1.56 ± 0.04^{a}
20% RG+80% HV	61.64 ± 1.80^{b}	2.75 ± 0.10^{b}	1.39 ± 0.04^{b}
P-value	**<0.001**	**<0.001**	**<0.001**
Additive			
Control	58.94 ± 0.77^{b}	2.60 ± 0.03^{b}	1.33 ± 0.02^{b}
Inoculant	66.23 ± 1.40^{a}	2.95 ± 0.07^{a}	1.50 ± 0.03^{a}
Molasses	66.29 ± 2.11^{a}	2.93 ± 0.09^{a}	1.50 ± 0.05^{a}
P value	**<0.001**	**<0.001**	**<0.001**
HV Level × Additive	**<0.001**	**<0.001**	**<0.001**
20% HV			
Control	60.30 ± 1.01^{b}	2.66 ± 0.05^{b}	1.37 ± 0.03^{b}
Inoculant	59.02 ± 1.89^{b}	2.60 ± 0.08^{b}	1.33 ± 0.05^{b}
Molasses	73.33 ± 3.11^{a}	3.24 ± 0.13^{a}	1.67 ± 0.08^{a}
P value	**<0.001**	**<0.001**	**<0.001**
40% HV			
Control	55.80 ± 0.60^{b}	2.46 ± 0.03^{b}	1.25 ± 0.01^{b}
Inoculant	66.88 ± 1.25^{a}	2.95 ± 0.05^{a}	1.52 ± 0.03^{a}
Molasses	60.14 ± 2.45^{b}	2.65 ± 0.11^{b}	1.35 ± 0.06^{b}
P value	**<0.001**	**0.001**	**<0.001**
60% HV			
Control	61.63 ± 1.90^{b}	2.71 ± 0.08^{b}	1.39 ± 0.05^{b}
Inoculant	69.92 ± 1.69^{ab}	3.08 ± 0.08^{a}	1.59 ± 0.04^{a}
Molasses	73.89 ± 0.19^{a}	3.26 ± 0.01^{a}	1.69 ± 0.00^{a}
P value	**<0.001**	**<0.001**	**<0.001**
80% HV			
Control	58.01 ± 0.66^{b}	2.56 ± 0.03^{b}	1.30 ± 0.02^{b}
Inoculant	69.10 ± 2.55^{a}	3.16 ± 0.16^{a}	1.58 ± 0.06^{a}
Molasses	57.81 ± 1.03^{b}	2.55 ± 0.05^{b}	1.30 ± 0.03^{b}
P value	**<0.001**	**<0.001**	**<0.001**

IVDOM: In vitro digestibility of organic matter; OM: Organic matter;
ME: Metabolic energy; NE$_L$: Net energy for lactation

did change the NDF and ADF ratios in the same ensiling times. NDF and ADF levels decreased with the increase in fermentation time. They expressed that this may be due to the growth of microorganisms and the development of fermentation. The effect of inoculant addition on NDF and ADF was also consistent with Özdüven et al. [30]. But Özdüven et al. [30] found that the effect of inoculant addition on sunflower silage IVDOM was not important, it resulted in just a numerical increase. The difference in the result from the presented study may be due to the difference in the silage material used. Similarly, Starczewski et al. [31] found that inoculant and molasses additives did not affect silage IVDOM. ME and NE$_L$ values of silages also changed similar to IVDOM.

The additives had no effect on the NDF and ADF rates. These results are consistent with those of Li et al. [29].

Table 3. Nutrient content of silages %DM.

	DM	Ash	OM	CP	NDF	ADF
M Level						
80% RG+20% HV	34.57 ± 0.72^{ab}	6.57 ± 0.54	93.43 ± 1.87	10.69 ± 0.98^{b}	46.09 ± 4.25^{b}	27.44 ± 2.25
60% RG+40% HV	37.84 ± 1.77^{a}	7.06 ± 0.37	92.94 ± 1.29	10.96 ± 1.32^{ab}	48.98 ± 3.96^{a}	29.12 ± 2.77
40% RG+60% HV	33.75 ± 0.50^{b}	6.60 ± 0.49	93.40 ± 1.69	11.87 ± 1.31^{a}	49.03 ± 2.45^{a}	28.92 ± 1.44
20% RG+80% HV	33.46 ± 0.37^{b}	6.45 ± 0.31	93.55 ± 1.09	11.08 ± 1.04^{ab}	46.65 ± 3.07^{b}	28.94 ± 1.98
P-value	**0.02**	**0.75**	**0.75**	**0.05**	**0.05**	**0.17**
Additive						
Control	34.86 ± 0.49	6.36 ± 0.27	93.64 ± 1.09	10.73 ± 1.17	48.53 ± 3.11	28.92 ± 1.82
Inoculant	34.03 ± 0.80	6.90 ± 0.50	93.10 ± 2.00	11.41 ± 1.15	47.21 ± 3.72	28.47 ± 2.20
Molasses	35.82 ± 1.36	6.75 ± 0.31	93.25 ± 1.25	11.32 ± 1.29	47.33 ± 4.13	28.41 ± 2.62
P value	**0.36**	**0.57**	**0.57**	**0.15**	**0.43**	**0.74**
HV Level × Additive	**0.54**	**0.17**	**0.17**	**0.04**	**0.03**	**0.07**
20% HV						
Control	34.73 ± 0.18	6.04 ± 0.48	93.96 ± 0.95	9.94 ± 1.18	50.77 ± 0.93^{a}	29.65 ± 0.49^{a}
Inoculant	35.04 ± 2.06	7.21 ± 1.18	92.79 ± 2.35	10.84 ± 0.60	43.94 ± 4.16^{b}	26.02 ± 1.94^{b}
Molasses	33.95 ± 1.12	6.45 ± 1.17	93.55 ± 2.35	11.30 ± 0.70	43.57 ± 2.05^{b}	26.65 ± 2.12^{b}
P value	**0.90**	**0.53**	**0.53**	**0.19**	**0.004**	**0.04**
40% HV						
Control	37.13 ± 1.26	6.24 ± 0.21	93.76 ± 0.41	10.26 ± 1.25^{b}	46.96 ± 3.34	27.53 ± 2.42
Inoculant	35.15 ± 2.26	8.49 ± 0.65	91.51 ± 1.31	12.10 ± 1.42^{a}	51.17 ± 1.76	30.63 ± 1.20
Molasses	41.22 ± 4.63	6.46 ± 0.19	93.54 ± 0.37	10.52 ± 0.42^{b}	48.81 ± 5.59	29.18 ± 3.80
P value	**0.06**	**0.07**	**0.07**	**0.04**	**0.18**	**0.11**
60% HV						
Control	34.48 ± 0.39	6.81 ± 0.86	93.19 ± 1.72	11.64 ± 0.64	49.89 ± 2.98	29.07 ± 1.84
Inoculant	32.58 ± 1.13	5.50 ± 0.95	94.50 ± 1.90	11.09 ± 1.38	47.70 ± 1.31	28.10 ± 0.85
Molasses	34.18 ± 0.78	7.48 ± 0.54	92.52 ± 1.07	12.89 ± 1.30	49.51 ± 2.79	29.58 ± 1.39
P value	**0.72**	**0.17**	**0.17**	**0.06**	**0.58**	**0.58**
80% HV						
Control	33.11 ± 0.43	6.33 ± 0.61	93.67 ± 1.22	11.07 ± 1.05	46.51 ± 3.11	29.43 ± 1.84
Inoculant	33.33 ± 0.69	6.40 ± 0.82	93.60 ± 1.64	11.61 ± 1.03	46.04 ± 3.13	29.13 ± 1.83
Molasses	33.94 ± 0.85	6.63 ± 0.15	93.37 ± 0.31	10.55 ± 1.03	47.40 ± 3.72	28.26 ± 2.58
P value	**0.94**	**0.96**	**0.96**	**0.38**	**0.83**	**0.70**

DM: Dry matter; OM: Organic matter; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre.

When the Hungarian vetch ratio increased to 40% and 60%, the NDF rate increased and decreased again to the level of 80%. ADF ratio did not change depending on both mixing ratios and additives. This situation is different from similar studies, and both NDF and ADF ratios decreased with the increase in legume ratio in similar studies [11,32]. Turan [11] stated that this decrease is due to the decrease in the amount of cell wall elements and the increase protein level of the mixture with the protein content of legumes. The CP and NDF changes obtained in the present study are not consistent with this explanation. The CP contents of silages did not increase in line with the increase in HV ratio, and it was observed that the additives did not affect the CP content as in similar studies [25,30]. Ash and OM contents of silages were not affected by different mixing ratios and additives. Among the mixture ratios, the DM level in the group containing 40% HV was found to be significantly higher than in other groups. It can be said that the DM content increases with the increase in the ryegrass ratio, as in the study of Kavut and Geren [32]. A

Table 4. Physical and sensory properties and Flieg scores.

	Odour (point)	Structure (point)	Color (point)	Total (point)	Flieg (point)
M Level					
80% RG+20% HV	12.83 ± 1.80	3.75 ± 0.62^{a}	1.75 ± 0.45	18.33 ± 2.35	100.03 ± 16.31
60% RG+40% HV	12.17 ± 2.33	3.17 ± 1.03^{ab}	1.58 ± 0.52	16.92 ± 3.85	103.87 ± 15.29
40% RG+60% HV	12.83 ± 2.17	3.58 ± 1.00^{ab}	1.83 ± 0.39	18.25 ± 3.42	87.46 ± 29.37
20% RG+80% HV	11.92 ± 2.71	2.83 ± 1.27^{b}	1.75 ± 0.45	16.50 ± 4.25	103.99 ± 11.48
P-value	**0.58**	**0.02**	**0.45**	**0.32**	**0.12**
Additive					
Control	13.25 ± 1.61^{a}	3.87 ± 0.50^{a}	1.94 ± 0.25^{a}	19.06 ± 2.11^{a}	103.05 ± 11.42
Inoculant	11.19 ± 2.51^{b}	2.88 ± 1.20^{b}	1.44 ± 0.51^{b}	15.50 ± 4.07^{b}	94.70 ± 27.38
Molasses	12.88 ± 2.06^{ab}	3.25 ± 1.07^{ab}	1.81 ± 0.40^{a}	17.94 ± 3.28^{ab}	98.76 ± 18.02
P value	**0.01**	**0.002**	**0.002**	**0.005**	**0.46**
HV Level × Additive	**0.10**	**0.001**	**0.11**	**0.03**	**0.19**
20% HV					
Control	13.00 ± 2.00	4.00 ± 0.00	2.00 ± 0.00	19.00 ± 2.00	106.26 ± 14.04
Inoculant	12.50 ± 1.92	4.00 ± 0.00	1.75 ± 0.50	18.25 ± 2.06	102.16 ± 24.99
Molasses	13.00 ± 2.00	3.25 ± 0.96	1.50 ± 0.58	17.75 ± 3.30	91.68 ± 1.86
P value	**0.92**	**0.27**	**0.19**	**0.83**	**0.53**
40% HV					
Control	13.00 ± 2.00^{a}	3.50 ± 1.00^{a}	1.75 ± 0.50^{a}	18.25 ± 3.50^{a}	107.66 ± 13.23
Inoculant	9.50 ± 1.00^{b}	2.00 ± 0.00^{b}	1.00 ± 0.00^{b}	12.50 ± 1.00^{b}	88.10 ± 5.53
Molasses	14.00 ± 0.00^{a}	4.00 ± 0.00^{a}	2.00 ± 0.00^{a}	20.00 ± 0.00^{a}	115.84 ± 10.49
P value	**0.01**	**0.002**	**0.002**	**0.002**	**0.11**
60% HV					
Control	13.00 ± 2.00	4.00 ± 0.00^{a}	2.00 ± 0.00	19.00 ± 2.00	101.56 ± 10.02
Inoculant	11.50 ± 3.00	2.75 ± 1.50^{b}	1.50 ± 0.58	15.75 ± 5.06	77.06 ± 45.08
Molasses	14.00 ± 0.00	4.00 ± 0.00^{a}	2.00 ± 0.00	20.00 ± 0.00	83.75 ± 24.54
P value	**0.22**	**0.03**	**0.12**	**0.11**	**0.18**
80% HV					
Control	14.00 ± 0.00^{a}	4.00 ± 0.00^{a}	2.00 ± 0.00	20.00 ± 0.00^{a}	96.72 ± 8.99
Inoculant	11.25 ± 3.40^{ab}	2.75 ± 1.50^{b}	1.50 ± 0.58	15.50 ± 5.45^{b}	111.49 ± 11.77
Molasses	10.50 ± 2.52^{b}	1.75 ± 0.50^{b}	1.75 ± 0.50	14.00 ± 3.27^{b}	103.78 ± 10.90
P value	**0.04**	**0.001**	**0.19**	**0.02**	**0.54**

similar situation in which the dry matter decreased due to the increase in the legume ratio was also observed in the studies of Demirel et al. [33] and Can et al. [34]. On the other hand, Li et al. [29] determined that DM loss decreased in the group containing inoculant and molasses after ensiling for 60 days. They attributed to the reduction of DM loss due to adequate fermentation and pH drop thanks to the substrate provided by molasses. Starczewski et al. [31] reported that inoculant and molasses additives did not affect the silage dry matter level as in this study.

While the odor and color of the silages were not affected by the different mixing ratios, except for structure, all of them were negatively affected by the inoculant additive. The structure was adversely affected by the increase in the proportion of HV, especially with the addition of inoculant. Contrary to these results, Karakozak and Ayaşan [10] stated that the addition of inoculants had a positive effect on silage quality. This may be because the inoculant used also contains enzymes. Aktürk and Gümüş [35] showed that the addition of inoculants had a positive effect on different ensiling periods as the ensiling period progressed. They stated that this is due to the increase in fermentation power during weeks and that the quality of fermentation may be affected by reasons such as the type of inoculant, nutritional value, and type of silage. Çetin and Arslan Duru [36] determined that 3% molasses addition had no effect on the odor, color, and structure of silage as in the presented study.

5. Conclusion

As a result, it was determined that Hungarian vetch can be mixed with ryegrass grass up to 80% with and without inoculant and molasses additive to obtain high-quality silage. But the highest digestibility values were obtained when Hungarian vetch was mixed at 60% level. It would be more appropriate to prefer molasses to avoid undesired changes in physical properties.

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References

1. Acar Z, Tan M, Ayan İ, Önal Aşçı Ö, Mut H et al. Türkiye'de yem bitkileri tarımının durumu ve geliştirme olanakları. In: Turkish Agricultural Engineering IX. Technical Congress Proceedings Book-1; Ankara, Turkey; 2020. pp. 529-553 (in Turkish)
2. Harmanşah F. Türkiye'de kaliteli kaba yem üretimi, sorunlar ve öneriler. Türkiye Tohumcular Birliği Dergisi 2018; 25: 9-13 (in Turkish).
3. Demiroğlu Topçu G, Özkan ŞS. General view to meadow-rangelands and forage crops cultivation of Aegean Region and Turkey (in Turkish). COMU Journal of Agriculture Faculty 2017; 5 (1): 21-28.
4. Abdi AM, Kılıç Ü. Effect of lignin peroxidase enzyme on feed values of different straws. Journal of Agriculture and Nature 2018; 21 (3): 374-384. doi:10.18016/ ksudobil. 346585 (in Turkish).
5. Yılmaz A, Yenice E, Yavaş İ, Çenesiz A. Hayvan beslemede mevcut durum ve gelecek. In: Turkish Agricultural Engineering IX. Technical Congress Proceedings Book-2; Ankara, Turkey; 2020. pp. 261-276 (in Turkish).
6. Şahin İF, Zaman M. An Important Cattle Feed: SILAGE (in Turkish). Doğu Coğrafya Dergisi 2010; 15 (23): 1-18.
7. Kurtoğlu V. Silaj ve silaj katkıları. Aybil Yayınları, Konya, 2011 (in Turkish).
8. Çakmakçı S, Aydınoğlu B, Arslan M, Bilgen M. Effect of different sowing methods on forage yield of common vetch (Vicia sativa L.) + perennial ryegrass (Lolium perenne L.) mixtures. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi 2005; 18 (1): 107-112 (in Turkish).
9. Acar Z, Bostan M. The effects of some natural additives on quality of alfalfa silage (in Turkish). Anadolu Journal of Agricultural Sciences 2016; 31 (3): 433-440. doi: 10.7161/ omuanajas.269998
10. Karakozak E, Ayaşan T. Effect of inoculant in silages in which different forage crops and their mixtures on Flieg point and crude nutrient content. Kafkas Üniversitesi Veteriner Fakakültesi Dergisi 2010; 16 (6): 987-994 (in Turkish).
11. Turan N. Determination of chemical composition and quality properties of silage produced by mixing green hungarian vetch and barley in varıous proportions. European Journal of Science and Technology 2019; 17: 787-793. doi: 10.31590/ejosat.643509 (in Turkish).
12. DLG. Energie- und Nahrstoffbedarf land wirtschaftlicher Nutztiere. 4. Schweine. DLG-Verlag GmbH, Frankfurt, Germany, 1987.
13. Polan CE, Stieve D, Garret JC. Protein preservation and ruminal degradation of ensiled forage treated with heat, formic acid, ammonia or microbial inoculant. Journal of Dairy Science 1998; 81 (3): 765-776. doi: 10.3168/jds.S0022-0302(98)75633-4
14. Filya I. The effect of Lactobacillus buchneri and Lactobacillus plantarum on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. Journal of Dairy Science 2003; 86 (11): 3575-3581. doi: 10.3168/jds.S0022-0302(03)73963-0
15. Tekin M, Kara K. The forage quality and the in vitro ruminal digestibility, gas production, organic acids, and some estimated digestion parameters of tomato herbage silage with molasses and barley. Turkish Journal of Veterinary and Animal Sciences 2020; 44: 201-213. doi: 10.3906/vet-1908-47
16. Barnett AJ. The colorimetric determination of lactic acid in silage. Biochemistry Journal 1951; 49: 527-529. doi: 10.1042/ bj0490527
17. AOAC. Official Method of Analysis, The Association of Official Analytical Chemists, 18th ed., Ed. WILLIAM HORWITZ, Maryland, Chapter 4, 2005.
18. Van Soest PJ, Robertson JB. Systems of analyses for evaluation of fibrous feed. In: Pigden WJ, Balch CC. and Graham M (editors). Proc. Int. Workshop on Standardization of Analytical Methodology for Feeds, International Development Research Center, Ottawa, Canada, 1979.
19. Goering HK, Van Soest PJ. Forage Fibre Analyses. Apparatus, Reagent, Procedures and Applications, USDA Agricultural Handbook No. 379, 1970.
20. Tilley JM, Terry RA. A two-stage technique for in vitro digestion of forage. Journal of British Grassland Society 1963; 18: 104-111.
21. Marten GC and Barnes RF. Prediction of energy digestibility of forages with in vitro rumen fermentation and fungal enzyme systems. In: Pigden WJ, Balch CC, Graham M (editors). Proc. Int. Workshop on standardization of analytical methodology for feed. International Development Research Center, Ottawa, 1979.

22. Weiss WP, Conrad HR, St. Pierre NR. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Animal Feed Science Technology 1992; 39: 95-110. doi: 10.1016/0377-8401(92)90034-4

23. Seydoşoğlu S. Investigation of the effect of fodder pea (Pisum sativum L.) and barley (Hordeum vulgare L.) herbages mixed at different rates on silage and feed quality. Ege Üniversitesi Ziraat Fakültesi Dergisi 2019; 56 (3): 297-302. doi: 10.20289/zfdergi.485698 (in Turkish).

24. Simić A, Vučković S, Kresović M, Vrbničanin S, Božić D. Changes of crude protein content in Italian ryegrass influenced by spring nitrogen application. Biotechnology in Animal Husbandry 2009; 25 (5-6): 1171-1179.

25. İnan Erbil N. The effects of homofermentative and/or heterofermentative lactic acid bacteria inoculants on the fermentation, and aerobic stability characteristics of hungarian vetch-wheat silages. Namık Kemal University, Graduate School of Natural and Applied Sciences, Master Thesis, 2012 (in Turkish).

26. Bingöl NT, Karslı MA, Bolat D, Akca İ. The effects of molasses and formic acid addition in sainfoin silage harvested at different maturities on silage quality and digestibility. Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi 2008; 2: 61-66 (in Turkish).

27. Bingöl NT, Karslı MA, Bolat D, Akca İ. Effects of molasses addition into barley-sainfoin mixture at varying levels on silage quality and digestibility. Atatürk Üniversitesi Veteriner Bilimleri Dergisi 2009; 4 (1): 23-30 (in Turkish).

28. Desta ST, Yuan X, Li J, Shao T. Ensiling characteristics, structural and nonstructural carbohydrate composition and enzymatic digestibility of Napier grass ensiled with additives. Bioresource Technology 2016; 221, 447–454.

29. Li Y, Du S, Sun L, Cheng Q, Hao J et al. (2022). Effects of Lactic Acid Bacteria and Molasses Additives on Dynamic Fermentation Quality and Microbial Community of Native Grass Silage. Frontiers in Microbiology 2022; 13: 1-13.

30. Özdüven ML, Tepeli C, Okuyucu B. The effects of lactic acid bacterial inoculants on the fermentation and aerobic stability of sunflower silages. Journal of Tekirdağ Agricultural Faculty 2017; 14 (02): 9-15.

31. Starczewski M, Purwin C, Borsuk M. Effect of various additives on the chemical composition, fermentation parameters and apparent digestibility of Virginia fanpetals silage in sheep. Journal of Elementology 2020; 25 (4): 1499-1508. doi: 10.5601/jelem.2020.

32. Kavut YT, Geren H. Effects of different harvest dates and mixture rates on the yield and some silage quality characteristics of legume mixtures with annual ryegrass (Lolium multiflorum L.). Ege Üniversitesi Ziraat Fakültesi Dergisi 2017; 54 (2): 115-124. doi: 10.20289/zfdergi.387050 (in Turkish).

33. Demirel R, Saruhan V, Baran MS, Andıç N, Şentürk Demirel D. Determination of different ratios of trifolium repens and hordeum vulgare mixtures on silage quality. Yüzüncü Yıl University Journal of Agricultural Sciences 2010; 20 (1): 26-31.

34. Can M, Kaymak G, Gülümser E, Acar Z, Ayab İ. Determination of silage quality of Bituminaria bituminosa with oat mixtures. Anadolu Journal of Agricultural Sciences 2019; 34 (3): 371-376. doi: 10.7161/omuanajas.548215.

35. Aktürk B, Gümüş H. Effects of lactic acid bacteria inoculant on quality, fermentation profile and nutritive value of alfalfa silage at different ensiling period. Ankara Üniversitesi Veteriner Fakültesi Dergisi 2020; 67 (3): 281-287.

36. Çetin İ, Aslan Duru A. Determination of some quality properties of ensiled forage turnip (Brassica rapa L.) with different additives. Fresenius Environmental Bulletin 2020; 29 (4A): 2766-2771.