

HUNGARIAN VETCH (*VICIA PANNONICA*) AND TRITICALE (*XTRITICOSECALE WITTMACK*) SILAGES, SOWED IN DIFFERENT SEED RATES, TREATED WITH LACTIC ACID BACTERIA + ENZYME MIXTURE INOCULANT: I. CHEMICAL COMPOSITION AND SILAGE FLEIG SCORE

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ABSTRACT

In this study, the effects of LAB+Enzyme inoculant (Sil All4x4, Altec, UK) added to triticale (T) and hungarian vetch (HV) silages planted at different seed rates (HV, 75:25 HVT, 50:50 HVT, 25:75 HVT and T) on the chemical composition and some silage properties were investigated. The research was carried out Gaziosmanpaşa University Agricultural Application and Research Center during the 2015-2016 vegetation period. Field experiment was designed as randomized block design with 3 replicates. The species and mixtures were harvested during the flowering period of the triticale. The crude protein concentration of experimental groups were 13.60%, 7.09%, 7.36%, 8.54% and 9.59% respectively. Besides, Lactic acid bacteria (LAB) + enzyme reduced crude protein and pH levels in silages. However, silage pH did not change among the experimental groups. But LAB+E application significantly reduced the pH level of silage (P<0.001). The addition of LAB+Enzyme to pure and mixed silages reduced pH, organic matter, crude protein, but increased NDF, crude ash and Fleig score. Also, the highest NEL value was obtained from 25HV+75T group. As a result, microbial and enzyme silage additives have positive effects on the fermentation properties of silage. As a result; by sowing hungarian vetch and triticale in a mixture; In terms of silage properties; gave better results than pure cultivation

Key words: Chemical composition, Hungarian vetch, triticale, LAB+Enzyme mixture, silage

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INTRODUCTION

In the production of quality roughage that animals need; mixing legumes with cereals is more advantageous than pure cultivation. Efficiency and quality of mixtures varies according to the species of cereals and legumes used in the mixture. In addition, mixing ratios of the plants used are the most important factors determining the feed efficiency and quality (Carr *et al.*, 1998). Hungarian vetch (*Vicia pannonica*) is cool season annual legume forage plant that can survive, grow at high altitudes, without freezing and damage, even in the harsh winters. The researchers stated that the most suitable harvest time of the Hungarian vetch and wheat mixture was between 30.1-36.7% of the dry matter of the mixture or during the milk maturity period of wheat (Aksoy and Nursoy 2010). On the other hand, triticale is an important cool climate grain species in human and animal nutrition due to its high grain and green forage yield, rapid growth and development, and high lysine content (Akgün and Kara 2002). Triticale is grown mostly for animal feeding as a grain product, sometimes for forage production or grazing as harvest and silage. Due to these properties of triticale, it is recommended to

be sown as a mixture in order to reduce the undesirable properties of Hungarian vetch when planted alone.

Physical, chemical and biological processing methods are used to increase the value of cellulose rich feeds. However, the improvement in the feeding value with these processing methods has not been able to exceed a certain level until this time. Therefore, the application of biological methods in this area has become more widespread today (Filya, 2007). Silage additives added to tropical forage crops silage increase the amount of lactic acid in silage and improve its quality (Lukkanukoll *et al.*, 2013). Regarding microbial inoculants, inoculant containing *Lactobacillus buchneri* alone or combination of *L. buchneri* and homo lactic acid can increase the aerobic stability of silages (Bernardes *et al.*, 2017). For ruminant animals to benefit more from roughages, studies have been carried out on lactic acid bacteria and fibrolytic enzyme applications that can break down plant cells in recent years. With these applications, long-term preservation and digestibility of both dry roughage and fresh and silaged roughage are provided (Adesogan *et al.*, 2014; Aboayge *et al.*, 2015). Filya (2001) in his study in corn silage; the LAB + enzyme mixture increased the fermentation properties of inoculant and maize silage while reducing their aerobic

stability. In addition, the application of LAB + enzyme mixture to silages decreased the ADF and NDF content of the silages and increased the rumen degradability of NDF and ADF (Filya 2001). Lactic acid bacteria and lactic acid bacteria + enzyme mixture inoculants increased the lactic acid level of sunflower silages while lowering the pH, acetic acid and NH₃-N levels. Also, Lab + E mixed inoculant decreased NDF content, increased organic matter and ADF digestibility (Özdüven *et al.* 2009). On the other hand, Yücel *et al.* (2013) stated that *L. buncheri* inoculant increases aerobic stability rather than contributing positively to silage quality.

In this study, the effects of LAB+Enzyme inoculants added to triticale and hungarian vetch silages planted at different seed rates on the chemical composition and some silage properties were investigated.

MATERIALS AND METHODS

Animal care and use: This study was carried out in accordance with the permission of the Animal Experiments Local Ethics Committee dated 03.08.2015, numbered 5.187.986.3-113 (2015 HADYEK-38).

Climate and soil characteristics of the research area: This study was conducted in the experimental area of Tokat Gaziosmanpaşa University Agricultural Research

Center during 2015-2016 vegetation period. Soil of trial area clayey, fertile and soil salt (2S / cm), pH, lime (%), P₂O₅ (kg/ha) K₂O (kg/ha) Organic matter (%) were respectively 0.032, 7.47, 2.42, 2.04, 1523.2, 19.8. The data related to the climate characteristics of the trial area are given in Table 1. In this study, Ege beyazı variety of Hungarian vetch (*Vicia pannonica* Crantz.) and Tatlıcak-97 variety of triticale (X*Triticosecale* Wittmack) were used as plant material. The field experiment was designed in a Randomized Complete Block Design with three replications. The seeds of pure plant species were sown in the last week of October 2015 as Ege beyazı (HV) 80 kg/ha and 250 kg/ha Tatlıcak (triticale). Experimental groups were sown as 1) HV, 2) 75:25HVT, 3) 50:50HVT 4) 25:75HVT, 5) T. The seeds were hand-sown in 15 rows of 6 meters length to be opened with a marker, in 20 cm row spacing. Each plot was 3m x 6m (18 m²) and the net research area was 17x23m: 391m². The species and their mixtures were harvested during the flowering period of the triticale. At harvest, plants were left as 2 rows from the edge of each plot and 0.5 m from the plot heads as a side effect and harvested in a net 2.6 x 5 = 13 m² area in each plot. Also weed control was done by hand. After harvesting, the ratios of vetch and triticale of the experimental groups (75:25 HVT, 50:50 HVT and 25:75 HVT) were found to be 47.8-52.2, 36.6-63.4 and 46.9-53.1 respectively.

Table 1. Climatic characteristics of trial area.

Monts/Items	Precipitation (mm)	Long year av. (mm)	Average (°C)	Long years ave. (°C)
March	59.0	57.1	8.1	9.7
April	34.5	23.6	10.0	13.5
May	34.8	31.9	16.9	18.0
June	35.4	48.5	20.0	21.0
July	0.2	3.8	23.5	24.8
August	7.6	4.4	24.3	25.6
September	0.2	19.6	23.2	22.5
October	55.6	51.8	16.1	15.7
Average	227.3	240.7	17.8	18.9

Preparation of silages: After harvest, fresh material was cut to about 2 cm long and a Lab + E mixture (Sil All^{4x4}, Altec, UK) inoculant was applied. The inoculant mixture was consist of lactic acid bacteria (*Pediococcus acidilactici*, *Lacto bacillus plantarum*, *Streptococcus faecium*) and enzymes (Cellulase, amylase, hemicellulase and pentosanase). The concentration of LAB inoculant in water was 0.005 g/liter. After diluting 0.1 g of LAB + enzyme mixture with 20 ml of tap water, it was sprayed on 10 kg of fresh material laid on clean nylon and mixed well. Thus, 106 cfu g⁻¹ lab and enzyme mixtures were added to each silage group (Filya, 2001). In the control groups that were not treated with Lab+E, fresh water was sprayed with the same amount of water alone, mixed

well, placed in jars with a capacity of approximately 2 kg, such as Lab + E, and tightly sealed for 50 days (Filya, 2001).

Chemical analysis: After the silage containers were opened, 25 g of silage samples were placed in a beaker and 100 ml of pure water was placed on it, mixed and pH was measured (Hanna Instruments HI 99163 portable pH meter) after filtration. Silages were dried at 70 °C for 48 hours to determine the dry matter (DM) contents (AOAC 31.007). The feed samples were ground through a 1 mm sieve for chemical analysis. The ash content of the forage samples were found in the muffle furnace at 525 °C for 8 hours. Crude protein content of silage samples was

determined by multiplying the total nitrogen amount determined by Kjeldahl method with 6.25 coefficient. Also, NDF and ADF contents of the feed samples were determined by Ankom (Ankom 200 Fiber Analyzer. Ankom tech.) (AOAC, 2007).

Calculation: Fleig score of the silage was calculated with the formula below.

$$\text{Fleig Score} = 205 + (2 \times \text{silage dry matter, \%}) - 40 \times (\text{silage pH content}) \quad (1)$$

Silage fleig score, very good between 81-100, good between 61-80, Pleasant between 41-60, medium between 21-40 and between 20-0 is considered bad. Also, Net energy lactation (NEL) values of silage samples were calculated by the formulas given below.

$$\text{NEL, Mcal/kg} = 2.0575 - (0.0199 \times \text{ADF}) \quad (2)$$

(Hungarian vetch)

$$\text{NEL, Mcal/kg} = 2.149 - (0.0223 \times \text{ADF}) \quad (3)$$

(Hungarian vetch and triticale mixture)

$$\text{NEL, Mcal/kg} = 2.296 - (0.0257 \times \text{ADF}) \quad (4)$$

(Triticale)

Equations were used (NRC, 2007).

Statistical analysis: Variance analysis of data was performed in the MSTATC statistical package program, with the data obtained in, Split plots in randomized complete block design with 3 replicates. The main plots, species and mixtures (1. HV, 2. 75:25HVT, 3. 50:50HVT 4. 25:75HVT, 5. T) and sub plots were inoculant applications (1. LAB+E and 2. No LAB+E). As a mathematical model for the effects of pure and mixture groups;

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk} \quad (5)$$

This equality, Y_{ijk} : Observation value, μ : General average, a_i : Effect of research groups ($i = 1, 2, 3, 4, 5$), b_j : effect of LAB+ Enzyme treatment ($j=1,2$), $(ab)_{ij}$: LAB+Enzyme application interaction with species and mixtures, e_{ijk} : Used as error value. LSD comparison test was used to determine the difference between research groups.

RESULTS

Chemical composition: The chemical composition of pure and mixture forages at harvest is shown in Table 2. The dry matter content of HV silage was lower than the other silage groups ($P < 0.05$). Also, LAB+E application did not change the dry matter content of the silage (Table 3). It was observed that there were no differences among the experiment groups in terms of pH level, but LAB+E application significantly reduced the pH level of silage ($P < 0.001$). Hungarian vetch silage had the highest crude protein content among the experimental groups. It has been observed that as the rate of Hungarian vetch decreases in the mixtures, the crude protein rate decreases accordingly. The lowest crude protein content occurred in the triticale silage ($P < 0.001$). LAB+E application to pure and mixed silages significantly reduced ($P < 0.001$) the crude protein ratio of silages (Table 3).

Table 2. Chemical composition of pure and mixture forages at harvest.

Cultivar and mixtures	Seed rate %	DM %	CP %	ADF %	NDF %	Crude ash %
HV	100	26.2	13.1	29.2	45.3	7.52
HV: T	75:25	33.5	11.1	29.8	45.5	6.90
HV:T	50:50	33.6	10.1	29.2	41.9	7.42
HV:T	25:75	34.1	9.1	28.8	45.6	9.35
T	100	34.0	5.3	31.7	47.3	6.53
Average		32.3	9.7	29.7	45.1	7.54

Table 3. The effect of LAB + E applied to triticale and hungarian vetch mixtures silages on the chemical composition.

Items	Tretment	DM %	pH	CP %	NDF %	ADF %
Cultivar and mixtures		%		%	%	%
HV		26.59 ^b	4.05	13.6 ^a	48.61 ^c	46.68
75 HV+25 T		33.05 ^a	3.96	9.60 ^b	53.11 ^b	48.78
50 HV+50 T		34.23 ^a	4.05	8.50 ^c	52.70 ^b	48.96
25 HV+75 T		33.21 ^a	3.89	7.40 ^d	53.76 ^b	48.85
Triticale		33.24 ^a	4.02	7.10 ^d	58.07 ^a	50.11
LSD		3.01	---	0.99	3.86	----
LAB+E.						
Treatment	Control	32.23	4.17 ^a	9.50 ^a	51.91 ^b	49.01
	LAB+E	31.90	3.82 ^b	8.97 ^b	54.60 ^a	48.34
Cultivar x LAB+E						

HV	Control	26.23	4.16	14.02	47.22	47.27 ^d
	LAB+E	26.90	3.94	13.17	49.99	46.09 ^d
75 HV+25T	Control	33.51	4.13	9.95	51.99	49.65 ^{abc}
	LAB+E	32.59	3.78	9.22	54.24	47.90 ^{bcd}
50 HV+50T	Control	33.61	4.26	8.48	49.23	48.15 ^{abcd}
	LAB+E	34.84	3.83	8.60	56.18	49.77 ^{abc}
25 HV+75T	Control	33.76	4.02	7.38	53.22	50.20 ^{ab}
	LAB+E	32.67	3.76	7.34	54.30	47.50 ^{cd}
Triticale	Control	34.03	4.25	7.67	57.87	49.77 ^{abc}
	LAB+E	32.46	3.78	6.51	58.27	50.45 ^a
LSD		----	----	----	----	2.098
CV (%)		5.16	3.99	4.51	4.08	2.37
<i>p</i> value	Cult. and mix.	0.0023**	0.4092	<0.001***	0.0045**	0.3429
	Treatment	0.2952	<0.001***	0.0057**	0.0069**	0.1456
	Treat* ^a Mix.	0.8180	0.623	0.0999	0.1579	0.0468*

Differences between mean values shown with different letters in the same column are statistically significant. *:p<0.05; **:p<0.01; ***:p<0.001

Triticale had the highest NDF concentration in the experimental groups, while the Hungarian vetch contained the lowest level of NDF (Table 3). Due to the decrease in the ratio of Hungarian vetch in mixtures, the NDF concentration of the silage has decreased significantly (P<0.01). Also, treatment of pure and mixed silage with LAB + E significantly increased NDF concentration (P<0.01). However treatment of silage with LAB+E decreased ADF concentration in the 25HV+75T group. It was determined that interaction of silage groups with LAB+E was significant (P<0.05). Ash content of the Hungarian vetch silage was higher than other silage groups (P<0.001). In addition, it was found that LAB+E application increased the ash content of the 50HV+50T silage and the interaction of ash content and LAB+E application was important (P<0.05).

It was observed that the organic matter of Hungarian vetch was significantly lower than the triticale and mixtures (P<0.01). Also the LAB+E treatment to silages significantly reduced the OM of the silage, the most decrease in organic matter was in the 50HV+50T group, and the LAB+E interaction was important (P<0.05). Lactic acid bacteria+enzyme mixture significantly increased the fleig score of silages compared to the control group (P<0.001).

Although there was no difference between pure and mixed silages in terms of NEL values, the application of LAB + E inoculant decreased NEL value in triticale and 50HV: 50T groups and increased it in other experimental groups (Table 4). The interaction of NEL content and LAB+E application was important (P<0.05).

Table 4. The effect of LAB + E applied to triticale and hungarian vetch mixtures on some silage Properties.

Items	Treatment	OM	Ash	Fleig score	NEL
Cultivar and mix.		%	%		M cal/kg
HV		88.85 ^b	11.15 ^a	99.66	1.129
75 HV+25 T		92.52 ^a	7.48 ^b	113.37	1.061
50 HV+50 T		91.27 ^a	8.73 ^b	111.63	1.059
25 HV+75 T		92.48 ^a	7.52 ^b	116.05	1.059
Triticale		92.06 ^a	7.94 ^b	110.88	1.008
LSD		1.57	1.57	----	0.081
LAB+Enzym					
Treatment	Control	91.69 ^a	8.31 ^b	102.87 ^b	1.056
	LAB+E	91.18 ^b	8.82 ^a	117.77 ^a	1.070
LSD					
Cultivar x LAB+E					
HV	Control	88.67 ^d	11.33 ^a	91.19	1.117 ^{ab}
	LAB+E	89.03 ^d	10.97 ^a	108.12	1.140 ^a
75 HV+25T	Control	92.71 ^a	7.29 ^d	106.28	1.042 ^{cde}
	LAB+E	92.34 ^{ab}	7.66 ^{cd}	120.45	1.081 ^{abc}
50 HV+50T	Control	92.01 ^{ab}	7.99 ^{cd}	101.67	1.075 ^{bcd}
	LAB+E	90.53 ^c	9.47 ^b	121.60	1.042 ^{cde}

25 HV+75T	Control	92.70 ^a	7.30 ^d	112.15	1.029 ^{cde}
	LAB+E	92.26 ^{ab}	7.74 ^{cd}	119.94	1.090 ^{abc}
Triticale	Control	92.38 ^{ab}	7.62 ^{cd}	103.05	1.017 ^{de}
	LAB+E	91.74 ^b	8.26 ^c	118.71	0.999 ^c
LSD		0.207	0.653	-----	0.057
CV (%)		0.39	4.19	7.74	4.85
<i>p value</i>	Cult. and mixture	0.0019 ^{**}	0.0019 ^{**}	0.0881	0.067
	Treatment	0.0029 ^{**}	0.0029 ^{**}	<0.001 ^{***}	0.154
	Treat*Mix.	0.0175 [*]	0.0175 [*]	0.4173	0.049 [*]

Differences between mean values shown with different letters in the same column were statistically significant *:p<0.05; **:p<0.01; ***:p<0.001, CV: coefficient of variation

DISCUSSION

In general, the dry matter content of the plants belonging to the poaceae family is higher than the legume plants. In this study, it was found that the dry matter contents of silages obtained from triticale and the mixture of triticale hungarian vetch were higher than that of Hungarian vetch silage. Aykan and Saruhan (2018) stated that the dry matter of silages increased with the increase in the ratio of barley in the mixtures when sowing fodder

peas and barley together. Species and mixtures were harvested at the beginning of flowering of triticale. During this period, it was observed that the crude protein content of silages was the highest in hungarian vetch and the lowest in triticale silage. Crude protein is one of the most important quality features of forage crops. Similar results reported by Geren *et al.* (2008) and Bengisu (2019) that legume-grass mixed silage increased with the increase of the legume ratio, as well as the crude protein content in silage.

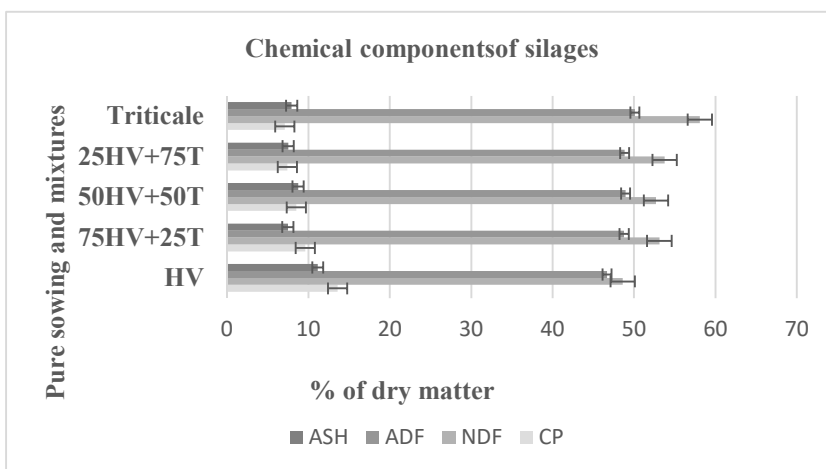


Figure 1. Chemical composition of silages from pure sowings and mixtures.

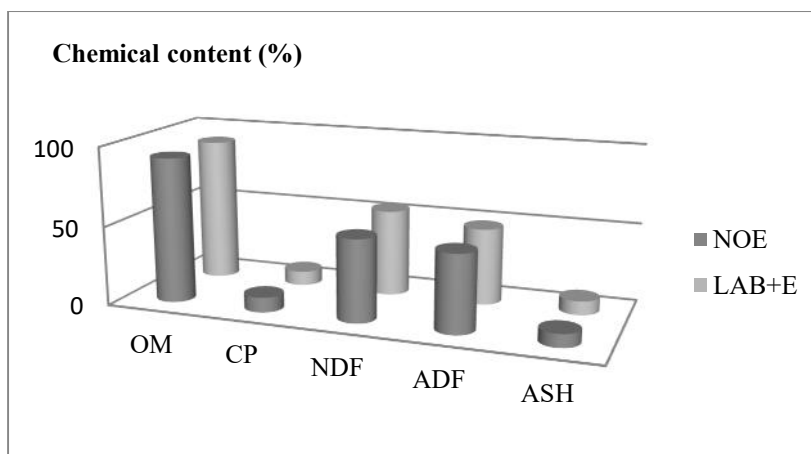


Figure 2. Effect of LAB+E on chemical content

In addition, Application of LAB+E Inoculants negatively affected the crude protein content of silages of species and mixtures. Loss of ammonia and other nitrogenous compounds can reduce CP content due to greater oxidation (Rotz and Muck, 1994). Similar results were reported by Arriola *et al.* (2010). However, Filya (2002) and Özdüven *et al.* (2009) stated that LAB+E use did not change the crude protein content in silage in their studies with maize, sunflower and triticale silages, respectively.

Silage pH value is one of the most important factors affecting silage quality (Ergun *et al.*, 2013). The pH values reflect the silage conditions of the material. Application of LAB + E to silage; Although it reduced the pH level of the silages, it was observed that there was no difference between the pH level of triticale, hungarian vetch and their mixtures. The nature of the fermentation process and the nutritional value of silage depend mainly on the feed characteristics and the types of microorganisms that dominate fermentation. It is stated that when forages are inoculated with homolactic bacteria before silage is made, the pH level of the resulting silage is generally lower than that without any application (Zahiroddini *et al.*, 2004)

Although the cell wall was at the lowest level in Hungarian vetch, it was observed that the NDF level increased with the increase in the ratio of triticale in the mixtures. This result was similar to stated by Bengisu (2019) that higher NDF values in cereals than in forage peas. Also, LAB+E application increased the NDF concentration of the silages by approximately 5%. Strydhorst *et al.* (2008) and Demirci *et al.* (2011) also stated that in their study with triticale and Hungarian vetch, homofermentative, heterofermentative bacteria and enzyme application increased NDF concentration in silage. Against that, Jones *et al.* (1992) noted that when bacterial inoculation was applied on alfalfa, the cell wall components changed and the protein and lignin associated with the carbohydrates of the cell wall were significantly reduced. Also, Similar results were declared by Lindsey and Kung, (2010) and Yücel *et al.* (2013).

Acid detergent fiber content refers to the amount of cellulose, lignin and insoluble protein in the cell wall structure of the plant cell (Carr *et al.*, 2004; Seydosoglu, 2019). ADF concentration of silages did not change in pure and mixed sowing. However, the application of LAB + E to silages decreased the ADF content in the 25HV + 75T group compared to the control. Similar results were reported by Torshizi *et al.* (2015). On the other hand, some researchers stated that the addition of LAB+E to silage material had no effect on ADF content (Özdüven *et al.* 2009; Demirci *et al.*, 2011; Keles *et al.*, 2014).

When pure and mixtures were harvested during the flowering period of triticale, organic matter of

hungarian vetch was lower than triticale and triticale+Hungarian vetch mixtures. Organic matter content was low as a natural consequence of the high mineral content during the harvest period of the Hungarian vetch. LAB+E inoculant reduced organic matter in silage compared to control group. As a result of LAB + E application to silages, it was determined that although the highest organic matter decrease was seen in 50HV+50T mixture group, the inoculant did not affect the OM content of the Hungarian vetch. This situation was thought to be due to the fact that the organic matter of the Hungarian vetch is lower than the other groups.

Crude ash content is an important feature as it gives an idea about the total mineral substance the plant contains. Crude ash content of Hungarian vetch silage was higher than mixtures an triticale. However, Aykan and Saruhan (2019) reported that the highest crude ash ratio (10.14%) was from a mixture of 50% pea + 50% barley, and the lowest crude ash ratio (8.17%) was from a mixture of 25% pea+75% barley. On the other hand, LAB+E application generally increased the ash content of silages. However, while the inoculant application did not change the ash content of the Hungarian vetch, it increased in the 50HV + 50T silage group. In addition, Demirel *et al.* (2010) stated that the mixture of alfalfa and barley containing 60% clover had the highest crude ash content (10.39%).

Although the difference between the research groups was not significant, the highest fleig score was obtained from 25% HV+75% T silage mixture. Similar results were reported by Seyidođlu (2019). In addition, Aykan and Saruhan (2019) observed that the Fleig score increased in parallel with the increase in barley ratio in mixtures. They stated that the highest Flieg score in the mixtures was 98.87%, and that they obtained from silage a mixture of 25% peas + 75% barley (variety of samyeli). Also similar results regarding fleig score of pure and mixed silage; Bengisu (2019) in triticale and barley mixtures; stated that 25H+75B mixture silage had the highest fleig score. The fleig score of silages treated with LAB+E inoculant was 13% higher. pH value used in the fleig scoring method is an important measure that numerically determines whether the feed is sour enough (Karakozak and Ayařan 2010). According to the results of many studies, a close relationship was determined between the fleig score and pH values (Kılıç, 1984; İptař and Avciođlu 1996). Also, 25HV + 75T mix group; It was determined that it had the highest NEL value among the experimental groups. However, Lithourgidis *et al.* (2006) stated that there was no difference in terms of energy values in vetch -triticale and vetch - oat pure and mixed cultivation.

Conclusion: The addition of LAB + E to pure and mixed silages reduced pH, organic matter, crude protein, but increased NDF, crude ash and Fleig score. The highest

NEL value was obtained from 25HV+75T group. Microbial and enzym silage additives have positive effects on the fermentation properties of silage As a result; by sowing hungarian vetch and triticale in a mixture; In terms of silage properties; gave better results than pure cultivation

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Conflict of Interest: The authors declare that they have no conflict of interest

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