



Determination of the silage quality of Lenox (*Brassica rapa*) product harvested in different periods

Farklı dönemlerde hasat edilen Lenoks (*Brassica rapa*) hasılının silaj kalitesinin belirlenmesi

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ABSTRACT

The objective of this study was to identify the impact of making silages using Lenox (*forage turnip, Brassica rapa* L.) harvested in two different stages of vegetation with bacterial inoculant+enzyme mixtures (0, 2.5, 5, and 7.5 mg.kg⁻¹) on the quality, in-vitro digestibility, and energy parameters of the silages. The vegetation period affected the examined variables significantly except for neutral detergent fiber (NDF), (p<0.005), and further into the vegetation period, dry matter (DM), organic matter (OM), ether extract (EE), NDF, acid detergent fiber (ADF), NH₃-N, pH, and Fleig score increased, whereas ash, crude protein (CP), lactic acid (LA), acetic acid (AA), and propionic acid (PA) decreased (p<0.005). The effect of additive levels added to the silages was found to be significant in terms of LA, AA, pH and fleig scores (p<0.05). The effects of the vegetation period and additives were found to be significant in terms of in vitro DM (DMD) and OM digestibility (OMD) and energy contents of Lenox silages (p<0.05). Further into the vegetation period, DMD and OMD values decreased. Compared to the control group, the greatest DMD, metabolic energy (ME), and net energy for lactation (NEL) results were found in the group with 2.5 mg.kg⁻¹ of additive. It was concluded that the VFA, DMD, OMD, SE, ME, and NEL results of the silage samples dropped as the vegetation period progressed, and when forage turnip was ensiled in its flowering period with the bacterial inoculant+enzyme mixture at a ratio of 2.5 mg.kg⁻¹, the quality of the obtained silages was high, and these can be utilized as alternative, quality roughage sources for feeding ruminants.

Key Words: Lenox, bacterial inoculant-enzyme, silage quality, *in-vitro* digestibility

ÖZ

Bu çalışma, iki farklı vejetasyon döneminde biçilen lenoks (*Brassica rapa* L.) bitkisinin, bakteriyel inokulant+enzim karışımı (0, 2.5, 5, and 7.5 mg.kg⁻¹) ile silolanmasının, silaj kalitesi, in-vitro sindirilebilirlik ve enerji içeriklerinin belirlenmesi amacıyla yürütülmüştür. Çalışmada, vejetasyon döneminin gruplar arasındaki farklılığa etkisinin nötr deterjan lif (NDF) değeri hariç, önemli olduğu (p<0.05); vejetasyon döneminin ilerlemesi ile kuru madde (KM), organik madde (OM), ham yağ (HY), NDF ve asit deterjan lif (ADF) içerikleri artarken, ham kül (HK) ve ham protein (HP) düzeylerinin azaldığı belirlenmiştir (p<0.05). Vejetasyon döneminin ilerlemesiyle, silaj LA, AA ve PA değerlerinin azaldığı; NH₃-N, pH ve Fleig puanı değerlerinin ise yükseldiği belirlenmiştir (p<0.05). Silajlara katılan katkı düzeylerinin etkisi, LA, AA, pH ve Fleig puanları bakımından önemli bulunmuştur (p<0.05). Lenoks silajlarının in-vitro KM (KMS) ve OM sindirilebilirlikleri (OMS) ile enerji içerikleri açısından, vejetasyon dönemi ve katkıların etkisi önemli bulunmuştur (p<0.05). Vejetasyon döneminin ilerlemesiyle, silajların KMS ve OMS değerleri azalmıştır. Silajlarda bakteriyel inokulant+enzim katkısı, kontrol grubu ile kıyaslandığında, en yüksek KMS, metabolik enerji (ME) ve net enerji laktasyon (NEL) değeri 2.5 mg.kg⁻¹ katkılı silaj

grubunda elde edilmiştir. Çalışmada, vejetasyonun ilerlemesine paralel olarak, silajların UYA, KMS ve OMS değerleri ile SE, ME ve NEL değerlerinin azaldığı; lenoks bitkisinin çiçeklenme döneminde biçilerek 2.5 mg.kg⁻¹ bakteriyel inokulant+enzim ile silolanması durumunda, elde edilen silajların kaliteli silaj niteliği taşıdıkları ve ruminantların beslenmesinde alternatif kaliteli bir kaba yem kaynağı olarak kullanılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Lenoks, bakteriyel inokulant+enzim, silaj kalitesi, *in-vitro* sindirilebilirlik

Introduction

Today, one of the most significant challenges faced by the animal husbandry sector is the provision of sufficient and balanced rations at a reasonable cost. Roughages constitute a large part of the rations of most ruminants and usually have low feed mass per volume. Most feeds in this group have high crude cellulose and low digestible energy contents. Pastures can be utilised as a source of roughage for only a few months per year. For this reason, roughages need to be harvested from pastures or cultivation areas, and they need to be stored in the form of grass, straw, or silage to meet roughage needs in seasons where some feed crops do not grow. Plants are generally reaped in harvesting periods with moisture contents of 60-75% and are ensiled directly. In ensiling, to achieve better fermentation, preservatives, fermentation stimulants or biological additives, other grains, or molasses are utilised in general (King, 2022; Yıldız et al., 2022, 2023).

To close the roughage deficit in the animal husbandry sector, it is necessary to increase the production of quality silages. This way, it will become possible to produce higher-quality and more robust silages that contain nutrients in amounts close to those of the initial material and provide them to animals. In addition to quality feeds, silage feeds must be cost-effective. If quality and water-rich silage feeds are utilized in the place of straw and fodder, which constitute the most prevalently preferred sources of roughage for ruminant animals, the use of concentrate feeds will be reduced, and there will be decreases in metabolic diseases and feed costs (Kılıç, 1986; Yaylak & Alçiçek, 2003).

Forage turnip (FT) is among the essential plants that are suitable for ensiling. FT (rapeseed, rapini;

Brassica rapa L.) is a Brassica species that grows prevalently in Anatolia and is potentially the Brassica species that was cultivated for the first time. Brassica species are frequently produced and utilised alternative feed crops for roughage needs in various regions of the world. Brassica species that are used as feed crop resources include FT (*Brassica rapa*), rapeseed (*Brassica napus* ssp. *oleifera*), and cabbage (*Brassica oleracea*) leaves. FT is an annual winter feed crop. For abundant yield outcomes in FT production, the end of October is the recommended sowing period. It is a high-yield feed crop that can be grown without any irrigation, provides a yield of 6-10 tons in a unit decare, is comparable to alfalfa as it contains 18-22% protein, 65-80% digestible DM, and approximately 20% NDF and 23% ADF, has wide leaves, and can grow as tall as 2.5 m. It has a significant place among feed crops that can be used by beef and dairy cattle farmers to lower the daily cost of feeds. FT provides abundant green fodder provides in a short time, and animals consume its forage fondly. Its digestibility ratio is very high. Under suitable circumstances, there is a short time between sowing and harvest maturity. While animals can be directly released to the land to graze in this maturity period, green fodder and silage-making are also options for plant use. Because of the short harvest maturity period of forage turnip, after-crop production is also possible (Kılıç, 2009; Doğan-Daş & Denek, 2021; Yıldız et al., 2022, 2023). FT can be ensiled after wilting or mixing it with other feed crops.

This study aims to evaluate the impact of harvesting the FT plant during its flowering and encapsulation periods and ensiling it with bacterial inoculant-enzyme mixtures at varying concentrations on the quality, IVD, and energy contents of silage products.

Material and Methods

Materials

FT was procured from the Research and Application Farmland of Van Yüzüncü Yıl University as the plant to be used in the study, whereas the bacterial inoculant and enzyme (Sil-All 4x4. Lallemand Animal Nutrition UK Ltd) were bought from a market in Van.

Methods

Making silage

The 2x4 factorial trial designs were utilized to perform the study. Forage turnips (FT) were collected in two different stages of vegetation (flowering period and encapsulation period). The plants were used without additives (control- 0 mg.kg⁻¹), and with 2.5, 5, and 7.5 mg.kg⁻¹ bacterial inoculant-enzyme (BIE) in weight-based mixtures, and 40 silage samples in total were stored in 1-litre glass jars. Following the piercing of the jar lids, the water in the jars was drained for 48 hours by keeping the jars upside down. After this process, the punctures on the lids were covered using duct tape. Following incubation for 70 days, the jars were opened.

Chemical analysis

Right after opening the silage containers, the pH values of the fluid part of the silages were determined using a digital pH measurement device. After drying at 65 °C for 48 hours, all samples were ground to a particle size of 1 mm in a laboratory-type mill (Nursoy & Şahin, 2017; Yıldız et al., 2022). Using the Weende analysis system (AOAC, 2019), the DM, CP, EE, and Ash values of the silages were analysed. The technique outlined by van Soest et al. (1991) was used for the ADF and NDF analyses. The NH₃-N concentrations of the silage liquids were determined by distillation (Anonymous, 2024). An HPLC device with the organic acid column branded Agilent Hi-Plex was utilised to study the silage sample liquids for AA, LA, PA, and BA (Suzuki & Lund, 1980).

Determination of Fleig scores, in-vitro digestibility,

and energy content of silages

Fleig score, as reported by Kılıç (1986), was calculated with the formula;

$$\text{Fleig Score} = 220 + (2 \times \% \text{DM} - 15) - 40 \times \text{pH} \quad (1)$$

The DMD and OMD values of the silages for *in-vitro* conditions were found using an Ankom Daisy II Incubator with the formula shown below (Ankom, 2022). The rumen fluid used in this study was obtained from a slaughterhouse in Van. It was collected fresh from the rumens of slaughtered cattle, quickly brought to the laboratory, and introduced into the device.

In vitro Digestibility:

$$\%(\text{IVD}) = 100 - ((W3 - (W1 \times C1)) \times 100) / W2 \quad (2)$$

W1: Filter bag weight, W2: Sample weight, W3: Final weight after NDF analysis, C1: Sample-free bag prepared for correction.

The formulas provided by NRC (2021) and Ishler et al. (2016) were utilised to determine the energy contents of the samples.

DE = Digestible Energy, Mcal.kg⁻¹ DM

$$\text{DE} = \text{TDN}\% (\text{OMD}) \times 0.04409 \quad (3)$$

ME, Metabolic Energy, kcal.kg⁻¹ DM

$$\text{ME} = \text{DE} \times 0.082 \quad (4)$$

NE_L, Net Energy Lactation, Mcal.kg⁻¹ DM

$$\text{NE}_L = (\text{TDN}\% (\text{OMD}) \times 0.0245) - 0.12 \quad (5)$$

Statistical analysis

The data collected in the study were subjected to statistical analyses with the analysis of variance (ANOVA) method, accompanied by Duncan's test of multiple comparisons as the post hoc test. For the analyses, the SPSS program was employed.

Results and Discussion

It is highly important to use quality and alternative roughages that are not only inexpensive but also unsuitable for consumption by humans to feed ruminants. In this sense, the FT plant is important thanks to its favourable

properties. This study investigated the effects of harvesting the FT plant in its flowering and encapsulation periods and ensiling it with BIE mixtures at varying ratios on silage quality, nutrient contents, energy contents, and *in vitro* digestibility.

The pre-ensiling nutrient parameter values of

the FT are presented in Table 1. As displayed by values included in Table 1, no significant variation was found in terms of pre-ensiling OM, CA, CP, NDF, or ADF values among the groups. As FT maturity levels increased, the dry matter and ether extract contents of the mixtures increased.

Table 1. Nutrients (DM, %) of the FT before ensiling

Period	Additive	DM	OM	Ash	CP	EE	NDF	ADF
Flowering Period	Control (0 mg.kg ⁻¹)	19,34	93,39	6,61	6,12	0,93	55,01	41,23
	2,5 mg.kg ⁻¹	19,38	94,35	5,65	6,86	0,78	51,41	38,97
	5 mg.kg ⁻¹	19,59	93,19	6,81	6,50	0,97	55,38	42,44
	7,5 mg.kg ⁻¹	19,85	93,45	6,55	7,20	1,39	55,96	41,47
Encapsulation Period	Control (0 mg.kg ⁻¹)	23,36	94,26	5,74	6,10	1,35	55,52	40,76
	2,5 mg.kg ⁻¹	23,33	93,97	6,03	6,67	1,19	53,22	40,65
	5 mg.kg ⁻¹	23,42	93,75	6,25	6,82	1,08	51,18	38,54
	7,5 mg.kg ⁻¹	23,25	93,45	6,55	6,35	1,37	57,13	42,97

CP: crude protein, DM: dry matter, OM: organic matter, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber.

In their FT study, Daş (2019) determined pre-ensiling values of DM, Ash, CP, ADF, and NDF in silage materials harvested in the encapsulation period as 18.06%, 8.81%, 10.35%, 38.71%, and 42.14%, respectively. Compared to the values found for the encapsulation period in this study, the results presented by Daş were higher in terms of CA and CP and lower for DM, NDF, and ADF contents. It is thought that the differences originated from the differences between the regions where these plants were grown. In another study, the ADF contents of FT leaves were reported as 18.34-19.74%, while their NDF contents were reported as 21.84-23.50% (Türk et al, 2009). The reported values were smaller than those calculated in the present study. The fact that only the leaves of the plants were used in the study conducted by Türk et al. may explain this difference. In a study that examined the yield properties of some FT (*Brassica rapa* L.) varieties, the ash and protein contents of the leaves of the plant were found to be 12.53-21.56% and 10.18-11.61% (Ayan et al., 2006). These results were greater in comparison to those obtained in this study. The fact that only the leaves of the plants

were used in their study may explain this difference. According to Çetin (2017), the Ash, CP, NDF, ADF, and DM results of the FT harvested at the end of the flowering period were, respectively, 23.33%, 10.04%, 19.96%, 36.04%, and 26.98%. Compared to the parameter results reached in our study, the values reported by Çetin were lower in terms of NDF and ADF, higher in terms of CA and CP, and similar in terms of DM.

When the nutrient parameter values of the FT silage samples are considered (Table 2), the vegetation stages had a significant influence on variations among the treatments, except for the NDF and ADF parameters ($p<0.001$). While the DM, OM, EE, NDF, and ADF values rose as the vegetation period progressed, the ash and protein values dropped ($p<0.001$). The additives significantly influenced the variations among the treatment groups for the Ash, NDF, and ADF values and had insignificant effects for the DM, OM, CP, and EE values ($p<0.0001$). As the ratio of the BIE mixture in the silages increased, no significant change was found in the nutrient content values of the silages. Furthermore, the period x interaction effects on the nutrient contents of these silage

samples were insignificant, except for NDF.

Table 2. Nutrients (DM, %) of the silage samples

Period	n	DM, %	OM, %	Ash, %	CP, %	EE, %	NDF, %	ADF, %	
Flowering Period	16	18.40±0.07b	93.12±0.15b	6.97±0.13a	7.70±0.17a	0.98±0.09b	51.92±0.92	39.92±0.76	
Encapsulation Period	16	22.05±0.33a	93.87±0.10a	6.17±0.09b	6.91±0.09b	1.86±0.42a	53.20±0.82	40.25±0.58	
P-value		0.000	0.000	0.000	0.001	0.000	0.109	0.506	
Additive									
Control (0 mg.kg ⁻¹)	8	20.10±0.73	93.13±0.26	6.87±0.26a	7.00±0.27	1.12±0.18	55.39±0.74a	42.32±0.76a	
2.5 mg.kg ⁻¹	8	20.57±0.80	93.55±0.24	6.76±0.19a	7.51±0.23	1.40±0.32	50.16±0.40c	38.35±0.48b	
5 mg.kg ⁻¹	8	20.49±0.75	93.55±0.18	6.45±0.18b	7.35±0.21	1.49±0.25	51.26±0.57bc	38.62±0.95b	
7.5 mg.kg ⁻¹	8	20.30±0.74	93.77±0.17	6.23±0.17b	7.35±0.24	1.67±0.19	53.70±0.67ab	41.28±0.43a	
P-value		0.112	0.085	0.011	0.335	0.060	0.002	0.001	
Period × additive		0.665	0.637	0.518	0.720	0.062	0.027	0.054	
Flowering Period	Control	4	18.23±0.38	92.58± 0.64	7.42± 0.64a	7.31±0.88	0.77±0.18b	56.41±1.69a	43.50±1.86a
	2.5 mg.kg ⁻¹	4	18.46±0.19a	93.25± 0.81	7.15±0.19ab	7.82±0.80	0.71±0.17b	50.00±1.17ab	37.97±0.61c
	5 mg.kg ⁻¹	4	18.55±0.32	93.13± 0.38	6.78±0.38ab	7.72±0.68	0.87±0.12b	48.62± 1.28c	37.36±0.35c
	7.5 mg.kg ⁻¹	4	18.38±0.10	93.52± 0.12	6.48± 0.12b	7.93±0.42	1.50±0.16a	52.23±1.08b	40.52±1.47b
	P-value		0.423	0.162	0.039	0.641	0.000	0.000	0.000
Encapsulation Period	Control	4	21.97±0.58	93.67± 0.19	6.33± 0.19	6.70±0.55	1.46±0.48	54.03± 1.51	40.76± 0.80
	2.5 mg.kg ⁻¹	4	22.68±0.30	96.84± 0.47	6.38± 0.20	7.20±0.28	2.09±0.29	50.27± 1.13	38.63± 1.67
	5 mg.kg ⁻¹	4	22.43±0.45	93.97± 0.18	6.03± 0.18	6.99±0.10	2.11±0.37	53.90± 5.09	39.88± 3.55
	7.5 mg.kg ⁻¹	4	22.22±0.60	94.02± 0.62	5.98± 0.62	6.76±0.11	1.84±0.78	54.81± 1.30	41.85± 0.44
	P-value		0.269	0.623	0.358	0.160	0.348	0.179	0.227

a, b, c: Different superscripts of mean values in the same column indicate significant difference (p<0.05). CP: crude protein, DM: dry matter, OM: organic matter, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber.

In a study that was carried out by adding wheat by 5% to three different *Brassica* species black mustard, canola and FT (Kılıc & Erisek, 2019), the Ash, CP, DM, EE, NDF and ADF. values of the FT silages prepared without additives were 22.11%, 10.12%, 1.90%, 7.76%, 61.16%, and 53.05%, respectively. In contrast with those obtained in the present study, these results were higher. The differences in the vegetation periods may have caused this difference. Daş (2019) showed the silage, ash, crude protein, dry matter, neutral detergent fibre and acid detergent fibre parameters of the FT plant harvested in the full encapsulation period and ensiled without additives, respectively, as 18.17%, 7.97%, 9.83%, 50.24%, and 46.61%. In comparison to the results of this study that were obtained from the FT silage prepared with the plants harvested in the encapsulation period without additives, the dry matter and neutral detergent fiber values stated by Daş were smaller. In contrast, the ash, crude protein, and acid detergent fiber values in their study were greater. The reason for this difference

may be the fact that these two studies were carried out with plants growing in different geographies. In another study (Özkan, 2019), for FT silages prepared without additives, dry matter, ash, crude protein, ether extract, neutral detergent fiber and acid detergent fiber values were reported to be respectively 23.18%, 9.65%, 12.53%, 2.92%, 41.05%, and 27.62%. The reported results were greater compared to those obtained with the silages that were prepared without additives in this study for dry matter, ash, crude protein, and ether extract, while they were lower than the ones in this study for neutral detergent fibre and acid detergent fibre. The differences in the vegetation periods may have caused this difference. Balakhial et al., (2008), who ensiled canola at a dry matter level of 20%, identified the dry matter, organic matter, crude protein, ether extract, neutral detergent fiber and acid detergent fiber contents of the silages as 17.82%, 88.00%, 15.68%, 6.00%, 52.33%, and 32.33%, respectively. Compared to those in this study, these values were higher for crude protein

and ether extract and lower for dry matter, organic matter, neutral detergent fibre and acid detergent fibre. Using two different *Brassica* species in these studies could be the reason for this difference. Another study, which involved the use of Hungarian vetch-triticale mixture silages as controls and in combination with bacterial inoculant, enzyme, and BIE additives (Can, 2010), reported DM-CP values of respectively 33.64%, 34.22%, 34.28%, and 34.13%-13.69%, 13.70%, 13.33%, and 13.66% in the milk stage and 40.90%, 41.24%, 40.91%, and 41.39%-12.41%, 13.08%, 12.06%, and 11.87% in the dough stage. Can stated that the bacterial inoculant and BIE mixture additives positively affected silage quality, and their results were close to those in this study. In yet another study (Filya, 2002), the effects of silage inoculants, including lactic acid bacteria and lactic acid bacteria-enzyme additives, on maize silage were investigated. Compared to the control group, it was determined that the additives reduced the NDF and ADF contents and raised the DM and CP contents of the silage samples. Their results were compatible with the results in this study.

As seen in Table 3, which presents the

fermentation-related parameter values of the silages, vegetation stages affected the variation among the groups significantly in terms of $\text{NH}_3\text{-N}$, lactic acid, acetic acid, pH, and Fleig scores and insignificantly in terms of propionic acid. Further into the vegetation process, the silage samples' LA, AA, and PA results dropped, whereas their $\text{NH}_3\text{-N}$ values, pH values, and Fleig scores rose. The silage samples had pH values within the optimal range. Regarding the fermentation parameters, the effects of the BIE additives used in this trial were not significant for $\text{NH}_3\text{-N}$ and PA but important for the LA, AA, pH, and Fleig score parameters. The BIE additive increased the silage samples' LA, AA, and pH values compared to the control group, while lowering the silage's $\text{NH}_3\text{-N}$ levels. All silage samples had pH values within the optimal range. The highest Fleig score was found in the silage that included 2.5 mg.kg^{-1} of additive, while the Fleig scores of all silages in the trial were in the "very good" category. It was also seen that the additive x vegetation period interaction had significant effects on the $\text{NH}_3\text{-N}$, pH, and Fleig score values of the silage samples (Table 3).

Table 3. Fleig scores and Fermentation quality of the FT silages

Period	n	NH ₃ -N Mg.dL ⁻¹	LA, %	AA, %	PA, %	pH	Fleig scores	Qualifications class	
Flowering Period	16	44.93±1.97b	4.88±0.14a	0.40±0.02a	0.03±0.00	3.67±0.02b	91.48±0.73b	Excellent	
Encapsulation Period	16	49.41±2.46a	3.29±0.09b	0.32±0.02b	0.02±0.01	3.87±0.02a	94.95±0.88a	Excellent	
P-value		0.007	0.000	0.002	0.232	0.000	0.001		
Additive									
Control (0 mg.kg ⁻¹)	8	49.37±3.38	4.45±0.47a	0.44±0.04a	0.03±0.01	3.86±0.02a	90.80±1.82b	Excellent	
2.5 mg.kg ⁻¹	8	45.17±0.75	4.06±0.26b	0.33±0.01b	0.01±0.00	3.78±0.02b	95.14±1.01a	Excellent	
5 mg.kg ⁻¹	8	45.17±2.89	3.84±0.25b	0.33±0.03b	0.03±0.01	3.79±0.03b	94.28±0.67a	Excellent	
7.5 mg.kg ⁻¹	8	48.83±5.55	3.87±0.48b	0.33±0.03b	0.03±0.02	3.82±0.04ab	92.65±0.99ab	Excellent	
P-value		0.073	0.000	0.013	0.087	0.005	0.010		
Period X Additive		0.000	0.001	0.052	0.037	0.001	0.016		
Flowering Period	Control	4	55.39±5.46a	4.77±0.20bc	0.53±0.04a	0.04±0.01	3.86±0.04a	87.05±2.46b	Excellent
	2.5 mg.kg ⁻¹	4	44.41±2.38b	5.49±0.31a	0.35±0.03b	0.02±0.01	3.74±0.01b	92.52±0.47a	Excellent
	5 mg.kg ⁻¹	4	38.96±5.62b	4.35±0.34c	0.35±0.05b	0.03±0.01	3.72±0.02b	93.39±0.97a	Excellent
	7.5 mg.kg ⁻¹	4	40.97±5.41b	4.92±0.19b	0.37±0.02b	0.03±0.01	3.72±0.01b	92.95±0.52a	Excellent
	p-value		0.002	0.006	0.000	0.138	0.000	0.000	
Encapsulation Period	Control	4	43.36±9.35b	3.52±0.21a	0.33±0.02	0.02±0.01b	3.86±0.06	94.54±4.27	Excellent
	2.5 mg.kg ⁻¹	4	45.94±1.83b	3.41±0.14a	0.32±0.01	0.01±0.00b	3.82±0.01	97.77±0.72	Excellent
	5 mg.kg ⁻¹	4	51.38±4.67b	3.32±0.15a	0.32±0.08	0.04±0.02a	3.87±0.04	95.16±2.30	Excellent
	7.5 mg.kg ⁻¹	4	64.56±9.72a	2.81±0.14b	0.30±0.10	0.03±0.02ab	3.93±0.08	94.95±3.51	Excellent
	P-value		0.021	0.002	0.925	0.046	0.116	0.183	

a, b, c: Means with different superscripts in the same column are significantly different ($p < 0.05$). $\text{NH}_3\text{-N}$: ammonia nitrogen, LA: lactic acid, AA: acetic acid, PA: propionic acid, pH: power of hydrogen.

Kılıç & Erisek (2019) studied the effects of adding wheat on the quality of silage samples produced using some plants in the Brassica genus (mustard, field mustard, and canola) and their IVTD (*in vitro* true digestibility) values. In the FT silage without any additive, the authors reported lactic acid, acetic acid, pH values, and Fleig scores of 1.16%, 2.22%, 5.05, and 32.11, respectively. The pH and acetic acid results obtained by Kılıç & Erisek for the silages they produced without any additive were greater than those in this study. At the same time, they reported lower lactic acid values and Fleig scores. The differences in the vegetation periods may have caused this difference. In their study performed on the FT, Daş (2019) stated the pH, $\text{NH}_3\text{-N}/\text{TN}$, lactic acid, acetic acid, and propionic acid values of silages without additives, respectively as 4.55%, 10.32%, 3.69%, 3.18%, and 0.05%. The pH and acetic acid values reported by Daş (2019) were higher in comparison to the ones found in this study, while Daş reported lower $\text{NH}_3\text{-N}$, lactic acid, and propionic acid values. The reason for this difference may be the fact that these two studies were carried out with plants growing in different geographies. Özkan (2019) presented fermentation values for FT silages, including pH, $\text{NH}_3\text{-N}$, lactic acid, acetic acid, and Fleig scores, as 4.20, 74.60, 5.72%, 2.91%, and 83.36, respectively. The pH, $\text{NH}_3\text{-N}$, lactic acid, and acetic acid results that Özkan found were greater than those found in the present study, while their Fleig scores were smaller. The differences in the vegetation periods may have caused this difference. In another study (Çetin, 2017), as fermentation parameters of FT silages without additives, pH, LA, AA, PA values, and Fleig scores of respectively 3.80, 1.98%, 0.23%, 0.16%, and 108.05 were obtained. Compared to the values in this study, these values were similar for pH, lower for LA and AA, and higher for PA and Fleig scores. In another study that

Can conducted (2010), for control, bacterial inoculant additive, enzyme additive, and BIE mixture additive groups of silages obtained with Hungarian vetch-triticale mixtures, pH values were found respectively as 4.07, 3.88, 4.13, and 3.93 in the milk stage and 4.28, 4.16, 4.27, and 4.10 in the dough stage, $\text{NH}_3\text{-N}$ values were found respectively as 75.39, 59.71, 68.32, and 44.69 in the milk stage and 74.16, 48.12, 58.21, and 52.95 in the dough stage, LA values were found respectively as 36.08%, 42.47%, 37.29%, and 39.48% in the milk stage and 29.64%, 36.16%, 32.51%, 34.90% in the dough stage, and AA values were found respectively as 13.18%, 9.93%, 10.13%, and 10.50% in the milk stage and 19.93%, 10.58%, 13.18%, and 12.15% in the dough stage. It was stated that the bacterial inoculant and BIE mixture additives positively affected silage quality, and their result was similar to those in this study. The effects of LA bacteria inoculants and enzymes on common vetch, wheat, and oat mixture silages were investigated in another study (İke, 2019). In the study, the bacterial inoculants and enzymes lowered the pH values and $\text{NH}_3\text{-N}$ contents of the common vetch, wheat, and barley mixture silages significantly, and in parallel with the results of this study, increased silage quality.

Considering the IVDMD and IVOMD results for the silage samples in the present study, the impact of the vegetation stages and additives was determined to be statistically significant (Table 4). Further in the vegetation period, the silages had decreased DMD and OMD values. These effects were also seen in the samples' DE, ME, and NEL values. In comparing the control group and the silages in which the BIE mixture additives were added, the silages with the 2.5 mg.kg^{-1} additive had the highest DMD, ME, and NE_L results. The period x additive interaction effects on the IVD and energy content results of the silages were found to be insignificant for all

silage groups.

Table 4. *In vitro* digestibility and energy content of silages of FT (DM, %)

Period		N	DMD	OMD	DE Mcal.kg ⁻¹ DM	ME kcal.kg ⁻¹ DM	NEL. Mcal.kg ⁻¹
Flowering Period		20	52.62±0.89a	56.25±0.87a	2.48±0.04a	2.03±0.03a	1.26±0.02a
Encapsulation Period		20	46.56±0.63b	50.48±0.72b	2.23±0.03b	1.83±0.03b	1.12±0.02b
P-value			0.000	0.000	0.000	0.000	0.000
Additive							
	Control (0 mg/kg)	10	46.24±1.06c	58.32±1.23ab	2.53±0.05ab	1.93±0.04ab	1.19±0.03ab
	2.5 mg.kg ⁻¹	10	51.84±1.17a	55.95±1.44a	2.47±0.06a	2.02±0.05a	1.25±0.04a
	5 mg.kg ⁻¹	10	51.50±1.50a	52.10±1.67b	2.30±0.07b	1.88±0.06b	1.16±0.04b
	7.5 mg.kg ⁻¹	10	48.78±1.65b	51.57±1.54b	2.27±0.07b	1.86±0.06b	1.14±0.04b
P-value			0.000	0.038	0.038	0.038	0.038
Period X Additive			0.102	0.920	0.920	0.920	0.920
Flowering Period	Control	5	47.87±1.25b	55.86±1.30	2.46±0.06	2.02±0.06	1.25±0.03
	2.5 mg.kg ⁻¹	5	54.75±1.19a	58.45±1.64	2.58±0.07	2.11±0.06	1.31±0.06
	5 mg.kg ⁻¹	5	54.92±1.15a	55.57±2.25	2.45±0.10	2.01±0.08	1.24±0.04
	7.5 mg.kg ⁻¹	5	52.96±1.00a	54.95±1.62	2.42±0.07	1.99±0.06	1.23±0.04
P-value			0.003	0.524	0.524	0.524	0.524
Encapsulation Period	Control	5	44.62±1.40b	50.78±0.70ab	2.24±0.06ab	1.84±0.05ab	1.12±0.01ab
	2.5 mg.kg ⁻¹	5	48.93±0.65a	53.44±1.72a	2.36±0.07a	1.93±0.06a	1.19±0.04a
	5 mg.kg ⁻¹	5	48.08±0.70a	49.50±1.03b	2.18±0.04b	1.79±0.03ba	1.09±0.02b
	7.5 mg.kg ⁻¹	5	44.60±0.67b	48.20±0.92b	2.13±0.03b	1.74±0.03b	1.06±0.02b
P-value			0.005	0.040	0.040	0.040	0.040

a, b, c: Means with different superscripts in the same column are significantly different (p<0.05). DMD: dry matter digestibility, OMD: organic matter digestibility, DE: digestible energy, ME: metabolic energy, NE: net energy lactation.

In another study (Kılıç & Erisek, 2019), where the effects of additives on the quality of silage samples produced using some plants in the *Brassica* genus (mustard, field mustard, and canola) and their IVD values were investigated, the IVD value of the FT silage without additives was determined as 54.89%. This higher value compared to those obtained in this study may be explained by the differences between the vegetation periods of the materials used in these two studies. In another study, in silage samples produced by the supplementation of field mustard with wheat straw and molasses at varying rates, the IVOMD and ME results of the control silages and silages with 10% straw addition were reported to be 50.18% and 50.07% and 7.69 MJ.kg⁻¹ and 7.63 MJ.kg⁻¹ dry matter, respectively (Daş, 2019). The *in vitro* organic matter digestibility and metabolic energy results provided Daş were lower than those found in this study. It may be stated that this difference was caused by the fact that the plants in the two studies were harvested in different vegetation periods, and the additives used were different. In

a study where the effects of a lactic acid bacteria inoculant-enzyme combination silage additive on silages prepared with wheat harvested in milk and dough stages were examined (Başkavak et al., 2008), it was observed that the additive increased both the DMD and OMD contents of the silage samples compared to the control group. These outcomes were similar to the results obtained with this study's 2.5 mg.kg⁻¹ BIE mixture additive. In a similar study (Kurşun, 2009), the effects of lactic acid bacteria inoculants on triticale fodder silages were examined, and the lactic acid bacteria inoculant-enzyme mixture additives increased the IVOMD values of the silages, as seen in this study.

Conclusion

In this study, it was concluded that the FT silages that were prepared by the addition of BIE mixtures were in the class of quality silages based on their fermentation quality values and Fleig scores, and they could be used as alternative, quality roughage sources to feed ruminant

animals. Considering the results of IVD and energy content, the highest-quality silages were obtained by adding the BIE mixture at 2.5 mg.kg⁻¹ to the FT plant that was harvested during the flowering period. Nevertheless, the results of similar studies may be more meaningful if the obtained OMD and energy values are compared against organic matter amounts obtained from the unit area.

Ethical Statement

No ethical permission was required to carry out this study.

Author contributions

SY conceptualised the study, developed the methodology, performed the experiments, validated the results, and wrote the original manuscript.

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Availability of Data and Materials

The data of this study are available from the authors upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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