SILAGE PERFORMANCE OF HOODED BARLEY GENOTYPES IN A SEMI-ARID AGROECOLOGICAL ZONE

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Abstract. The increasing demand for high-quality roughage necessitates silage-oriented cereal cultivars for ruminant productivity under variable climates. This study evaluated silage quality traits of 20 hooded barley lines, four standard barley cultivars, and one triticale cultivar over three growing seasons (2019–2021) in southeastern Türkiye. Parameters included dry matter (DM), crude protein (CP), fiber fractions (ADF and NDF), pH, and derived traits such as relative feed value (RFV). Statistical analyses included ANOVA, linear mixed models, Pearson correlation, and principal component analysis. Significant genotype and year effects were observed for most traits ($p < 0.0001$), except CP ($p = 0.125$). The highest DM contents were recorded in 2021 for barley (41.4%) and triticale (41.7%). CP ranged from 6.0% to 15.3%, with triticale showing the highest mean in 2021 (11.9%). ADF values ranged significantly from 27.2% (barley, 2020) to 41.1% (triticale, 2019). NDF ranged from 43.3% (barley, 2021) to 59.5% (triticale, 2019). RFV values spanned 73.1–189.7, with the highest in barley cultivars in 2021 (149.3) and the lowest in 2019 for both species (106.1–106.3). Linear mixed model analysis revealed significant yearly RFV increases of 16.5 units ($p < 0.001$) with substantial genotypic variance (49.33). PCA revealed antagonistic relationships between fiber traits (ADF, NDF) and energy traits (RFV, DM), while CP remained independent. These findings suggest that certain hooded barley genotypes possess superior silage quality traits, supporting their potential for forage breeding programs in arid and semi-arid conditions.

Keywords: *silage quality, hooded barley, fiber fractions, relative feed value, genotype × year interaction*

Introduction

Cereal grains are among the most extensively cultivated crops globally, serving as staple foods for millions and constituting a major component of animal feed (Adeboyajo et al., 2023). Currently, approximately one-third of global cereal production, around 800 million tons, is used as livestock feed, with this figure expected to exceed 1.1 billion tons by 2050 due to increased demand for animal protein (Makkar, 2018; Stewart and Lal, 2018). In Turkey, annual production of quality forage stands is about 29.6 million tons, yet this only satisfies approximately 37.6% of livestock requirements (for 17.1 million animal units), leaving a roughage deficit of nearly 49 million tons (Tan and Yolcu, 2021).

Access to high-quality forage is critically important for maintaining the productivity and health of ruminant livestock. Although livestock production in Turkey largely relies on natural pastures, animals are often fed with low-nutrient agricultural residues such as wheat stubble during dry periods, leading to significant losses in performance (Seydosoglu, 2019). Under such conditions, silage produced by fermenting high-moisture forages with lactic acid bacteria under anaerobic conditions offers a practical solution by preserving nutritional value and supporting animal productivity during periods of feed scarcity (Duniere et al.,

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2013). Barley (*Hordeum vulgare* L.) is globally the fourth most cultivated cereal (146 million tons produced in 2023), with 70% of its production used in animal feeding. Barley is favored in marginal environments due to its adaptability to drought, salinity, and disease, coupled with low production costs (Nikkhah, 2012; Elakhdar et al., 2022). In Turkey, barley's use extends beyond grain; its regrowth ability post-harvest and suitability for silage makes it an ideal candidate for filling national forage gaps (Nair et al., 2016).

Despite barley's advantages, most breeding and cultivation efforts focus on grain quality. There is a lack of emphasis on developing hooded barley varieties specifically optimized for silage. Awns pose health risks to livestock and complicate harvest and handling (Huang et al., 2021). Hooded phenotypes, resulting from specific genetic variations, offer safer, more efficient options for forage producers (Huang et al., 2020). This study evaluates silage quality traits of 20 hooded barley lines, four standard barley cultivars, and a triticale control, grown in the Harran Plain of Şanlıurfa, Turkiye. The aims are to: (1) compare nutritional quality among genotypes, including dry matter, fiber, protein, and fermentation parameters, (2) identify superior silage materials for forage production in the region, and (3) provide data to support breeding programs focused on forage-targeted barley lines. By focusing on hooded barley tailored for silage, this research addresses forage deficit in the region while enhancing ruminant nutrition. The findings are expected to inform both local agricultural strategies and breeding efforts aimed at improving forage quality without compromising grain yield.

Materials and methods

Experimental materials

This study utilized 20 hooded barley lines (38.7 g) (ICA-AWNS 011/13, /18, /22, /26, /32, /34, /38, /39, /41, /42, /45, /47, /50, /55, /56, /59, /62, /63, /67, /75), whose pedigree information and development history were provided by the GAP Agricultural Research Institute based on their promising yields in regional trials. Additionally, four standard barley cultivars Yerli Siyah (33.5 g), Akhisar 98 (41.9 g), Altıkat (35.6 g), and Kendal (33.2 g) and one triticale cultivar (Ege Yıldızı) were included for comparative analysis. While the primary aim of the study was to assess agronomic traits, a secondary, though unpublished, objective was to identify the most suitable lines and cultivars for silage quality in the region, with a view toward their incorporation into future breeding programs. The four barley cultivars were selected based on their known high forage yield potential, while the inclusion of the triticale cultivar was justified by its superior performance in both biomass and silage yield under local growing conditions. A comprehensive list of the genetic materials used in this research is presented in *Table 1.*

Study area, soil, and climatic characteristics

The experimental trials were conducted at the Serince Research Station located within the Şanlıurfa province, in Southeastern Anatolia of Turkiye. The soil at the experimental site is derived from alluvial parent material, has a reddish-brown color, and is classified as heavy clay in texture. These characteristics are typical of the region's irrigated agricultural zones and are highly relevant for forage and silage crop cultivation. The soil's high clay content and structure influence water retention and nutrient availability, which are critical factors in evaluating biomass and silage yield potential across genotypes. The measured parameters, including pH, electrical conductivity (EC), lime content, available phosphorus (P_2O_5), potassium (K_2O), nitrogen (N), organic matter, and key micronutrients (Cu, Fe, Mn, Zn), are presented in *Table 2*.

Table 1. *Genetic materials employed in the present study*

Row no	Lines and varieties		Row no	Lines and varieties	
1	ICA-AWNS 011/13	Hooded barley	14	ICA-AWNS 011/55	Hooded barley
2	ICA-AWNS 011/18	Hooded barley	15	ICA-AWNS 011/56	Hooded barley
3	ICA-AWNS 011/22	Hooded barley	16	ICA-AWNS 011/59	Hooded barley
4	ICA-AWNS 011/26	Hooded barley	17	ICA-AWNS 011/62	Hooded barley
5	ICA-AWNS 011/32	Hooded barley	18	ICA-AWNS 011/63	Hooded barley
6	ICA-AWNS 011/34	Hooded barley	19	ICA-AWNS 011/67	Hooded barley
7	ICA-AWNS 011/38	Hooded barley	20	ICA-AWNS 011/75	Hooded barley
8	ICA-AWNS 011/39	Hooded barley	21	Yerli Siyah	Two row barley
9	ICA-AWNS 011/41	Hooded barley	22	Akhisar 98	Six row barley
10	ICA-AWNS 011/42	Hooded barley	23	Altıkat	Six row barley
11	ICA-AWNS 011/45	Hooded barley	24	Kendal	Six row barley
12	ICA-AWNS 011/47	Hooded barley	25	Ege Yıldızı	Triticale
13	ICA-AWNS 011/50	Hooded barley			

Table 2*. Soil characteristics of the experimental area*

Parameter	pH	EC (ds/m)	Lime (%)	P_2O_5 (kg/da)	K_2O (kg/da)	N (%)	Organic matter (%)	Cu (mg/L)	Fe (mg/L)	Mn (mg/L)	Zn (mg/L)
Value	7.95	1.10	24.7	1.73	105.6	0.156	1.02	1.116	0.945	0.820	0.268

Şanlıurfa has a continental climate (hot/dry summers, cold/rainy winters). Climate data from the experimental years (2019-2021) differed from long-term averages (*Fig. 1*). Winters in 2019-2020 were colder than average, while spring 2021 was hot and dry. Severe drought occurred during the 2020-2021 growing season; April-June received very little or no rainfall. Low relative humidity exacerbated drought stress. Conversely, winter 2019-2020 had above average rainfall. These climatic differences significantly impacted barley development and yield. Climatic deviations of the experimental years (2019–2021) from the long-term average (1929–2022), Turkish State Meteorological Service – MGM, 2021) were displayed in *Table 3*.

Experimental design and cultural practices

This three-year field study (2019–2021) was conducted using a randomized complete block design (RCBD) with three replications for each measurement. Each experimental plot measured 6 m² and consisted of six rows, spaced 20 cm apart, with a row length of 5 m.

Exact sowing and harvest dates were as follows:

- 1st year: sowing on 20 January 2019, harvest on 20 April 2019
- 2nd year: sowing on 26 November 2019, harvest on 26 April 2020
- 3rd year: sowing on 23 November 2020, harvest on 23 April 2021

Sowing was carried out at a seeding rate of 180 kg per hectare, with seeds placed at a depth of 3–4 cm following standard soil preparation practices. Basal fertilization consisted of 60 kg ha^{-1} nitrogen (N) and 60 kg ha^{-1} phosphorus (P_2O_5), supplemented by an additional 80 kg ha^{-1} of nitrogen applied as a topdressing at the tillering stage.

Irrigation was applied to reduce drought stress: sprinkler irrigation was used once after sowing if rainfall was insufficient for emergence, and two additional irrigations were carried out during the growing period.

Weed management was performed mechanically, depending on weed pressure. Harvesting was conducted at the milk stage of maturity.

Silage preparation and laboratory analyses

After harvest, forage was chopped (5–7 cm) and packed into 1.5-L glass jars, then stored at room temperature in a dark environment for 60 days for fermentation. The top 3–5 cm of silage was discarded before analysis. pH was measured from a 1:10 (w/v) silage extract using a WTW 7310 digital pH meter (Polan et al., 1998). Dry matter (DM) was determined by drying 25-gram samples at 65°C for 48 h, followed by precision weighing (AOAC, 2005). Crude ash, crude protein (CP), Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF) were analyzed using air-dried, 1 mm-sieved silage samples via Near-Infrared Reflectance Spectroscopy (NIRS), calibrated with the C-0904FE-Hay and Fresh Forage standard (Hoy et al., 2002).

Table 3. *Monthly average climatic values for the experimental years and long-term averages (MGM, 2021)*

Month	Years	Minimum temp. (°C)	Maximum temp. (°C)	Average temp. (°C)	Average relative humidity (%)	Total precipitation (mm)
November	2019	3.70	20.40	11.20	42.30	0.90
	2020	4.82	18.29	10.56	63.83	54.20
	Long-term*	8.50	18.8	13.10	63.50	44.70
December	2019	3.30	11.60	7.00	79.40	141.10
	2020	1.08	13.15	6.27	61.85	13.50
	Long-term	4.00	12.00	7.60	69.10	80.20
January	2020	0.20	9.20	4.40	69.10	38.70
	2021	0.56	13.01	5.86	60.04	63.80
	Long-term*	2.10	9.90	5.60	71.50	87.40
February	2020	0.20	11.00	5.1	63.40	38.30
	2021	0.25	15.87	7.52	54.34	1.00
	Long-term*	2.90	12.00	7.10	67.70	68.30
March	2020	4.50	17.40	10.50	63.60	64.10
	2021	2.21	16.88	9.25	55.53	37.0
	Long-term*	5.80	16.40	10.90	64.40	62.80
April	2020	6.20	22.90	14.00	54.20	28.10
	2021	8.38	25.54	17.08	45.29	0.50
	Long-term*	10.30	22.30	16.20	62.80	49.50
May	2020	11.20	28.80	20.00	41.00	6.10
	2021	12.30	32.57	23.12	33.28	0.00
	Long-term*	15.30	28.80	22.30	54.10	26.50
June	2020	16.20	34.70	26.00	29.90	6.20
	2021	15.83	34.47	25.77	34.85	0.00
	Long-term*	20.10	34.70	28.10	40.90	4.30

*MGM, (1929-2022)

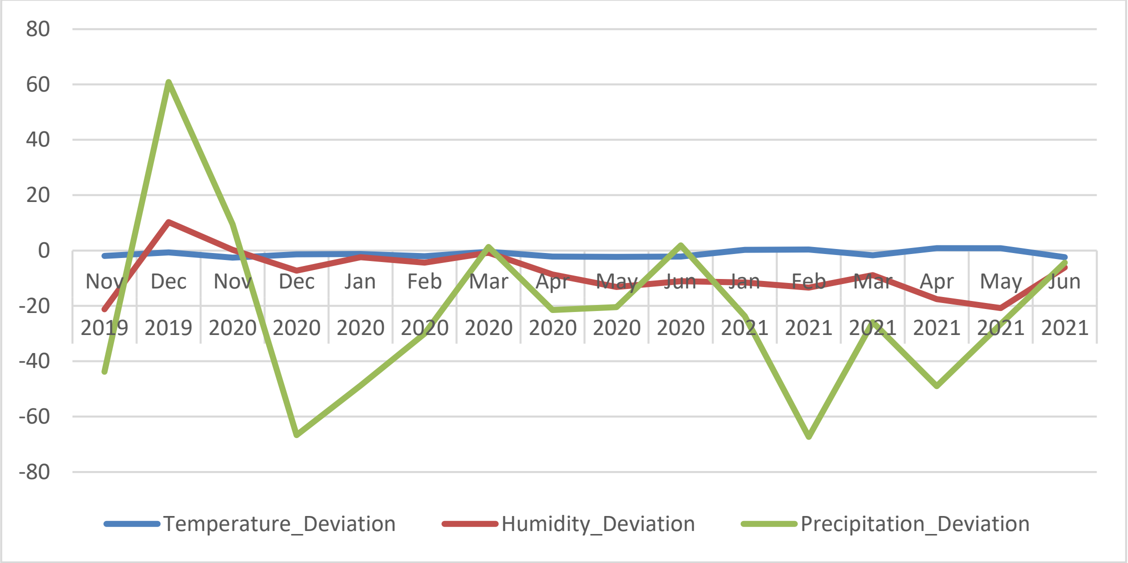

Figure 1. *Climate deviations from long-term averages.*

Additional forage quality parameters were calculated:

$$\text{Digestible dry matter (DDM, \%)} = 88.9 - (0.779 \times \%\text{ADF}) \qquad \text{(Eq.1)}$$

$$\text{Dry matter intake (DMI, \% of body weight)} = 120 / \%\text{NDF} \qquad \text{(Eq.2)}$$

$$\text{Relative feed value (RFV)} = (\text{DDM} \times \text{DMI}) / 1.29 \text{ (Rohweder et al., 2002)} \qquad \text{(Eq.3)}$$

DDM = digestible dry matter; ADF = acid detergent fiber; DMI = dry matter intake; NDF = neutral detergent fiber; RFV = relative feed value.

Statistical analyses

Principal component analysis of quality traits

Principal Component Analysis (PCA) was employed to reduce the dimensionality of the multivariate silage quality data and to explore patterns among genotypes and years based on quality traits. The variables included were pH, Dry Matter (DM), Crude Protein (CP), ADF, NDF, and Relative Feed Value (RFV). The dataset consisted of 75 observations derived from 25 genotypes across three years (2019–2021).

All variables were standardized using z-score normalization to eliminate scale effects. PCA was conducted using the sklearn. decomposition. PCA module in Python. The first two principal components (PC1 and PC2) were retained, as they captured the majority of total variance. Biplots were constructed to visualize genotypic distribution (score plot) and trait contribution (loading plot) within the principal component space.

Lmm analysis for RFV in silage quality study

A linear mixed model (LMM) was applied to evaluate the effects of year and genotype on Net Relative Feed Value (RFV). The model treated “Year” as a fixed effect and “Genotype” as a random intercept to account for repeated measures across years. The analysis was conducted using the “statsmodels” package in Python (v0.14.0) with restricted maximum likelihood estimation (REML). Model assumptions were verified through residual diagnostics, and significance was assessed at $p < 0.05$.

Correlation test

Pearson correlation analysis was conducted to examine the interrelationships among six major silage quality traits: pH, Dry Matter (DM), Crude Protein (CP), Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), and Relative Feed Value (RFV). Data were collected from 25 genotypes evaluated over three consecutive years (2019–2021), yielding a combined dataset suitable for correlation analysis.

All variables were checked for normality and homogeneity before analysis. The correlation matrix was generated using the Pearson method, which assesses linear relationships between variables. Visualization of the correlations was achieved through a heatmap, created using the seaborn library in Python. Correlation coefficients (r) were interpreted as follows: $|r| > 0.7$ as strong, $0.3 < |r| \leq 0.7$ as moderate, and $|r| \leq 0.3$ as weak.

Variance analysis

Analysis of variance (ANOVA) was performed to assess the effects of genotype, year, and their interaction on silage quality parameters, including pH, dry matter ratio, crude protein ratio, ADF, NDF, and relative feed value (RFV). Mean comparisons were conducted using Tukey’s Honest Significant Difference (HSD) test at the 5% significance level ($p < 0.05$) to determine significant differences among treatment means. All statistical analyses were conducted using ExcelStat, an advanced statistical analysis add-in for Microsoft Excel. The software was used for both ANOVA computations and post hoc multiple comparisons.

Results

The analysis of silage quality traits across all experimental materials, including 20 hooded barley lines, four standard barley cultivars, and one triticale cultivar, revealed considerable variation in several parameters *Table 4*. The pH values ranged from 3.5 to 7.7, with an overall mean of 5.1 and a standard deviation of 0.7. While most samples fell within the desirable range for well fermented silage, the upper extreme suggests that a few genotypes may have undergone suboptimal fermentation, potentially due to insufficient lactic acid production or high buffering capacity.

***Table 4**. Descriptive statistics of silage quality parameters across all genotypes evaluated (2019–2021)*

Variable	Minimum	Maximum	Mean	Std. deviation
pH	3.5	7.7	5.1	0.7
DM (%)	19.2	57.8	32.2	7.8
CP (%)	6.0	15.3	9.9	1.2
ADF	19.9	46.0	31.3	4.7
NDF	36.0	76.9	49.6	6.3
RFV	73.1	189.7	123.7	22.2

DM = Dry Matter Ratio (%); CP: Crude Protein Ratio (%); ADF: Acid Detergent Fiber (%); NDF: Neutral Detergent Fiber (%); RFV: Relative Feed Value

The dry matter ratio exhibited substantial variability, with values ranging from 19.2% to 57.8%, averaging 32.2% and showing a relatively high standard deviation of 7.8%. This indicates a wide range in moisture content across genotypes, which may influence both fermentation efficiency and silage stability. Samples with dry matter below 25% are at risk of undesirable fermentations, while excessively high values may lead to poor compaction and aerobic spoilage. Crude protein content was more stable across genotypes, with values between 6.0% and 15.3%, and a mean of 9.9%. The low standard deviation (1.2%) suggests that most entries maintained moderate protein levels, with a few standout genotypes reaching higher values that may be advantageous for ruminant nutrition, particularly in systems emphasizing dual purpose use.

Fiber-related traits showed more pronounced variation. Acid detergent fiber (ADF) ranged from 19.9% to 46.0%, with a mean of 31.3% and a standard deviation of 4.7%. Neutral detergent fiber (NDF) ranged from 36.0% to 76.9%, averaging 49.6% with a standard deviation of 6.3%. These results imply substantial differences in digestibility and voluntary intake potential among the evaluated materials, as higher ADF typically indicates lower digestibility and higher NDF may reduce intake.

Relative Feed Value (RFV) also varied widely, from 73.1 to 189.7, with an average value of 123.7 and a standard deviation of 22.2. This broad range highlights significant differences in the energetic value of the silage among the tested genotypes. Entries with RFV values near or above 150 may offer high energy density, particularly beneficial for lactating animals with elevated nutritional demands.

Genotypic and temporal variation in silage quality traits

The analysis of variance (ANOVA) revealed statistically significant differences among the genotype × year combinations for most silage quality traits *Table 5*. Significant effects were observed for pH ($p < 0.0001$), dry matter ratio ($p < 0.0001$), ADF ($p < 0.0001$), NDF ($p < 0.0001$), and RFV ($p < 0.0001$). However, crude protein ratio did not show a significant variation among the treatments ($p = 0.125$). Post hoc comparisons showed that the lowest mean pH value was recorded in the triticale cultivar in 2019 (4.16), although four other combinations showed statistically equal values, indicating that low acidity was not exclusive to triticale. In contrast, the highest mean pH value was observed in barley cultivars in 2020 (5.77) and was significantly higher than that of several other groups.

Regarding dry matter (%DM) content, the highest values were observed in the 2021 triticale and barley combinations (41.7% and 41.6%, respectively), while the lowest was recorded in the 2019 barley varieties (22.9%), indicating that dry matter content was significantly affected by both year and cultivar. Although crude protein content was not statistically significant across treatments, triticale variety for 2021 exhibited the highest mean crude protein ratio (11.9%), followed by triticale variety for 2020 (10.6%). Regarding ADF content, the highest value was observed in the 2019 triticale combination (41.1%), while the lowest values were recorded in the 2021 barley and triticale combinations (27.2–28.9%). These results indicate that ADF content was significantly influenced by both year and cultivar. A similar pattern was noted for NDF, where triticale variety for 2019 showed the highest mean value (59.5%), whereas barley varieties for 2021 had the lowest (43.330%). In terms of RFV, barley varieties for 2021 recorded the highest energy value (149.3), significantly outperforming other groups. The lowest RFV was observed in barley varieties for 2019 (106.1) and triticale variety at 2019 (106.3). While year × genotype interactions were tested, they were statistically significant only for ADF (p = 0.008) and NDF (p = 0.002). This suggests that the effect of genotype varied across years primarily for fiber-related traits, but not for other parameters.

Table 5. *Silage quality parameters of barley lines, barley varieties, and triticale across years (mean ± SD and ANOVA results)*

Material × year	pH	DM (%)	CP (%)	ADF (%)	NDF (%)	RFV
Barley lines × 2019	4.97 b*	25.7 cd	9.9 b	35.5 b	55.6 a	108.1 d
±SD	5.27 ± 0.66	26.33 ± 4.08	9.94 ± 0.86	35.35 ± 5.43	55.56 ± 4.95	106.08 ± 14.30
Barley lines × 2020	5.61 a	29.9 bc	9.9 b	31.2 cd	50.6 b	121.4 cd
±SD	5.56 ± 0.67	30.36 ± 2.23	9.71 ± 0.88	31.61 ± 3.39	50.77 ± 3.93	121.42 ± 11.79
Barley lines × 2021	4.76 bc	41.6 a	9.9 b	28.8 d	44.8 c	138.9 abc
±SD	4.84 ± 0.43	43.11 ± 4.37	9.53 ± 1.57	28.74 ± 3.64	44.91 ± 3.97	132.47 ± 16.07
Barley varieties × 2019	4.71 bc	22.9 d	9.9 b	29.1 d	46.6 c	106.1 d
±SD	4.82 ± 0.57	22.72 ± 3.16	9.91 ± 0.93	29.86 ± 4.25	46.86 ± 4.38	101.43 ± 13.24
Barley varieties × 2020	5.77 a	29.6 bc	9.9 b	27.2 d	45.1 c	125.7 bcd
±SD	5.77 ± 0.41	30.36 ± 2.26	9.96 ± 0.88	26.57 ± 3.18	46.63 ± 3.86	122.59 ± 8.77
Barley varieties × 2021	4.60 bc	41.4 a	9.3 b	27.6 d	43.3 c	149.3 a
±SD	4.91 ± 0.40	41.53 ± 4.22	10.92 ± 1.67	28.56 ± 4.04	44.74 ± 4.67	149.27 ± 20.15
Triticale × 2019	4.16 c	26.2 cd	10.0 ab	41.1 a	59.5 a	106.3 d
±SD	4.16 ± 0.60	26.16 ± 1.05	9.96 ± 0.86	41.11 ± 3.83	59.46 ± 3.00	106.30 ± 17.36
Triticale × 2020	5.39 ab	32.1 b	10.6 ab	34.0 bc	52.6 ab	136.9 abc
±SD	5.39 ± 0.43	32.10 ± 1.63	10.62 ± 0.96	34.00 ± 5.67	52.64 ± 6.64	136.87 ± 14.42
Triticale × 2021	4.86 bc	41.7 a	11.9 a	28.9 d	44.8 c	143.8 ab
±SD	4.86 ± 0.43	41.70 ± 4.37	11.90 ± 1.57	28.90 ± 3.64	44.80 ± 3.97	143.80 ± 16.07
Pr > F (model)	<0.0001	<0.0001	0.125	<0.0001	<0.0001	<0.0001
Pr > F (lines and varieties × year)	0.158	0.465	0.179	0.008	0.002	0.437

*Values within the same column followed by different letters are significantly different according to Tukey's Honest Significant Difference (HSD) test at p < 0.05. DM = Dry Matter Ratio (%); CP: Crude Protein Ratio (%); ADF: Acid Detergent Fiber (%); NDF: Neutral Detergent Fiber (%); RFV: Relative Feed Value

Effects of year and genotype on silage quality traits: linear mixed model analysis

The linear mixed model revealed that "Year" had a statistically significant effect on RFV (p < 0.001), with an estimated increase of approximately 16.5 units per year. The variance attributable to genotype differences was also substantial (49.33), indicating strong genotypic influence on silage energy content. The intercept and slope estimates, along with their standard errors and significance values, are presented in *Table 6*.

Table 6. *LMM results for silage quality parameters*

Parameter	Intercept	Year coef	Intercept SE	Year SE	Intercept z	Year z	Intercept p	Year p	Genotype var	Residual var
pH	163.7	-0.08	112.79	0.056	1.45	-1.41	0.147	0.16	0.0	0.47
DM	-16432.2	8.15	610.19	0.302	-26.93	26.98	0.0	0.0	3.15	13.69
CP	52.8	-0.02	180.73	0.089	0.29	-0.24	0.77	0.81	0.27	1.20
ADF	6169.3	-3.04	616.47	0.305	10.01	-9.96	0.0	0.0	2.47	13.97
NDF	9968.4	-4.91	731.84	0.362	13.62	-13.55	0.0	0.0	3.44	19.69
RFV	-33240.9	16.52	2673.33	1.323	-12.43	12.48	0.0	0.0	49.33	262.72

DM = Dry Matter Ratio (%); CP: Crude Protein Ratio (%); ADF: Acid Detergent Fiber (%); NDF: Neutral Detergent Fiber (%); RFV: Relative Feed Value

The LMM results showed that most quality parameters were significantly influenced by year and also exhibited considerable variation across genotypes. The fixed effects of year were particularly strong for RFV and Crude Protein, indicating improving trends over time. Random effects indicated that genotypic variability contributed substantially to overall variance in all parameters. These findings suggest that breeding and genotype selection play a key role in optimizing silage quality under different environmental conditions.

Principal component analysis of silage quality traits

PCA was used to examine multivariate patterns in silage traits across genotypes and years. The score plot based on PC1 (45.20%) and PC2 (18.76%) shows genotype year combinations, with shapes for genotypes and colors for years. Clear year-based separation was observed: 2021 samples clustered negatively on PC1, associated with higher RFV and DM, while 2019–2020 samples were grouped positively, linked to higher fiber (ADF, NDF) and pH. PC2 reflected genotype level differences, mainly driven by protein (*Figs. 2* and *3*).

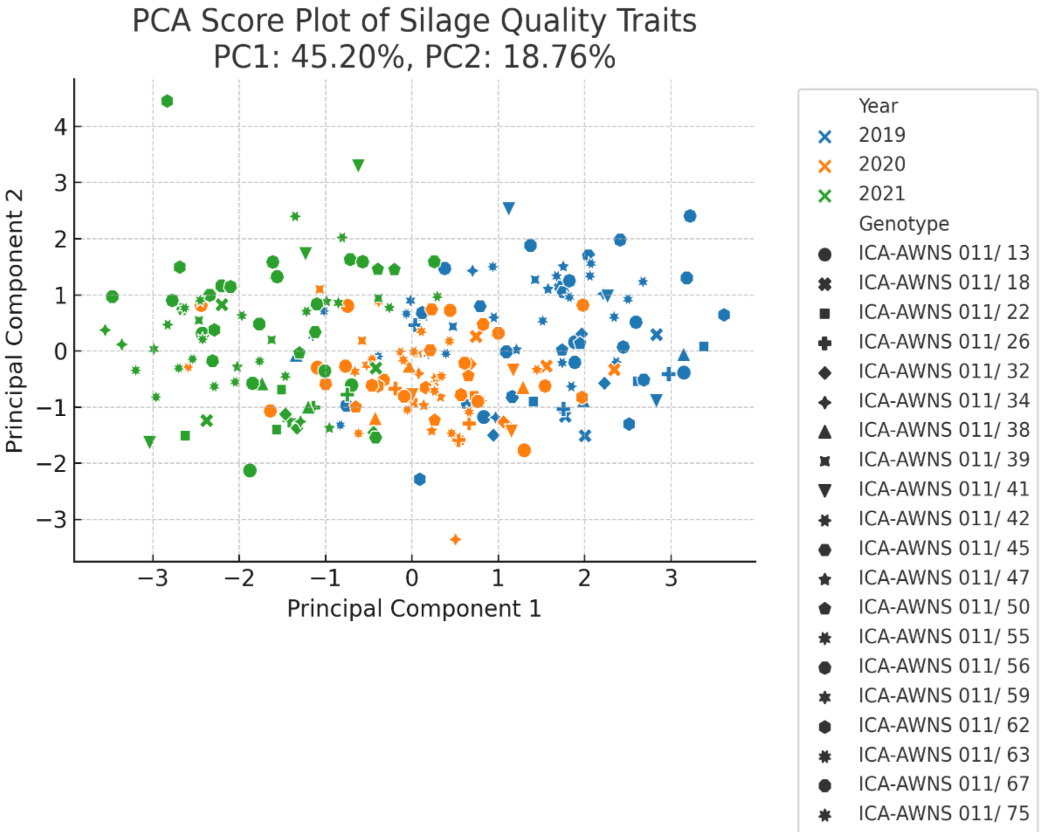

Figure 2. *PCA score plot of silage quality traits across years and genotypes*

PC1 was positively loaded by NDF (0.544), ADF (0.512), and pH (0.115), and negatively by RFV (–0.451) and DM (–0.474), representing a fiber to energy gradient. PC2 was positively influenced by protein (0.706) and negatively by pH (–0.667), indicating variation in fermentation and nitrogen content *Table 7*. Loading plot representing the contribution and orientation of individual silage quality traits in the PCA

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space. ADF and NDF load strongly and positively on PC1, while DM and RFV load negatively, confirming their inverse relationship in silage nutritional composition. Crude protein loads most strongly on PC2, orthogonal to the fiber-energy axis, indicating that crude protein variation is largely independent of structural carbohydrate and energy traits. The direction and magnitude of each vector reflect the trait's influence on sample distribution in the score plot.

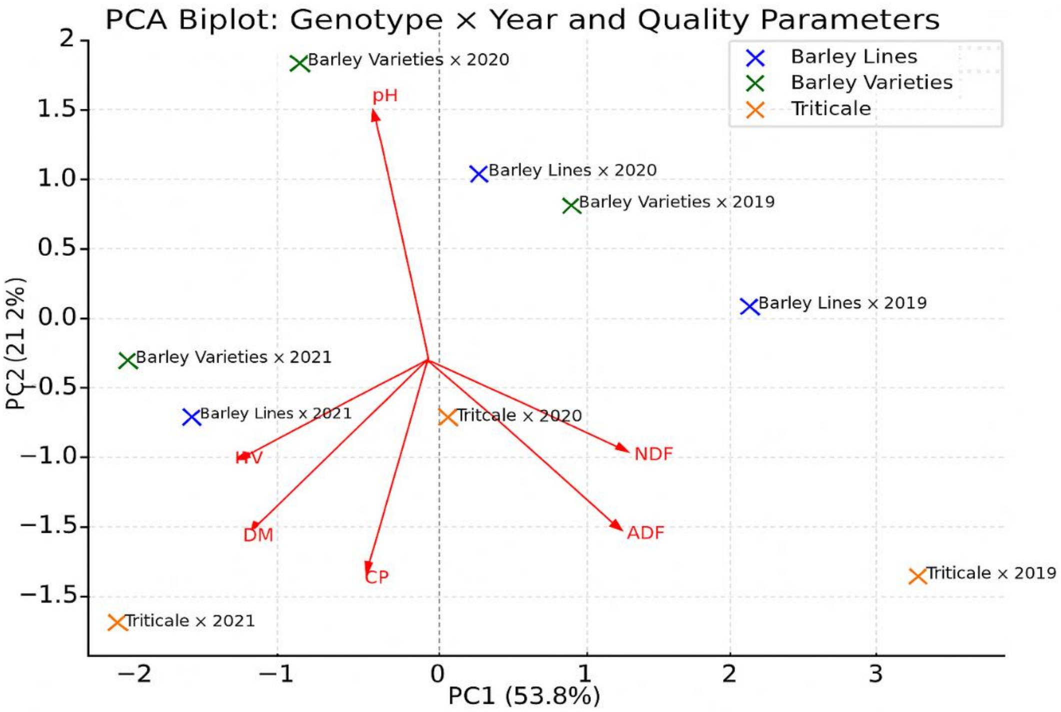

Figure 3. *PCA loading plot of silage quality parameters. DM = Dry Matter Ratio; ADF: Acid Detergent Fiber; NDF: Neutral Detergent Fiber; RFV: Relative Feed Value*

Table 7. *PCA loadings for silage quality traits*

Trait	PC1	PC2
pH	0.115	-0.667
DM	-0.474	-0.043
CP	0.024	0.706
ADF	0.512	0.137
NDF	0.544	0.084
RFV	-0.451	0.170

DM = Dry Matter Ratio (%); CP: Crude Protein Ratio (%); ADF: Acid Detergent Fiber (%); NDF: Neutral Detergent Fiber (%); RFV: Relative Feed Value

Correlation test results

The Pearson correlation matrix revealed several significant relationships. ADF and NDF showed a strong positive correlation (r = 0.87), indicating structural interdependence. RFV was moderately correlated with DM (r = 0.59), suggesting that higher dry matter is linked to greater energy density.

ADF and NDF were negatively correlated with RFV (r = –0.43 and –0.47), reflecting the typical fiber-energy trade off. DM also showed negative correlations with ADF (r = –0.43) and NDF (r = –0.56), indicating that lower fiber promotes higher dry matter levels.

Crude protein content showed weak or no correlation with other traits (|r| ≤ 0.13), suggesting independence from fiber and energy variables. Similarly, pH was largely uncorrelated, showing only a weak negative link with RFV (r = –0.16) (*Fig. 4*).

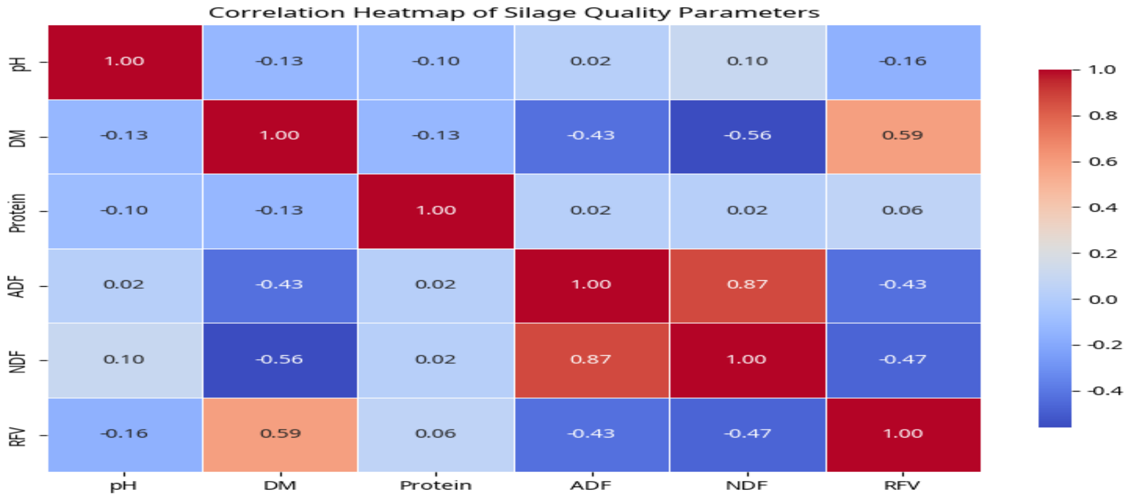

Figure 4. *Correlation heatmap of silage quality parameters. DM: Dry Matter Ratio; ADF: Acid Detergent Fiber; NDF: Neutral Detergent Fiber; RFV: Relative Feed Value.*

Discussion

Interpretation of silage quality parameters and genotype × year interactions

Silage quality traits, especially fiber and energy, showed significant genotypic and temporal variation. pH differences indicate fermentation efficiency variability (Muck, 2010). Optimal fermentation is typically associated with a rapid drop in pH to the range of 3.8–4.2, which inhibits undesirable microbial activity and promotes lactic acid dominance (Jatkauskas et al., 2024). Higher pH values observed in some barley samples from the 2020 season suggest less effective acidification, potentially leading to greater dry matter losses and reduced silage stability (Borreani et al., 2018). Conversely, the lower pH values recorded in triticale silage may reflect more efficient fermentation, possibly linked to higher water-soluble carbohydrate content or lower buffering capacity (Marković et al., 2011). The broad range of observed pH values highlights the interactive effects of genotype and growing season on silage fermentation dynamics. Elevated pH may indicate suboptimal activity of lactic acid bacteria or a high initial buffering capacity in the plant material both conditions which can compromise fermentation efficiency and result in nutrient losses (Muck, 2010; Kitaw et al., 2024). These findings emphasize the importance of selecting genotypes with favorable fermentation profiles and monitoring pre-ensiling factors that influence pH dynamics, such as water-soluble carbohydrate content and buffering capacity (Kitaw et al., 2024).

Dry matter content in silage is a critical determinant of both fermentation pathways and aerobic stability. Variations in DM arise not only from harvest timing and drying conditions but also from genotypic differences among forage crops. Optimal DM content (30–35%) ensures sufficient compaction to maintain anaerobic conditions and inhibit spoilage organisms (Kleinschmit et al., 2005). However, low DM content can foster Clostridium species, leading to butyric acid fermentation and significant energy loss (Xie et al., 2022). Conversely, excessively high DM hampers proper compaction, allowing oxygen infiltration that predisposes silage to aerobic spoilage, particularly through the growth of undesirable yeasts and Acetobacter spp. under air exposure (Bai et al., 2024).

Crude protein (CP) content did not differ significantly, but triticale consistently showed higher values, beneficial for ruminant diets (Cherney and Cherney, 2003). Forage species vary in protein, with triticale often having better profiles than some small grains (Cherney and Cherney, 2003). Significant ADF and NDF differences, and their year interactions, highlight environmental effects on cell wall composition (Hansey et al., 2010). High NDF/ADF generally means lower digestibility and dry matter intake (Nair et al., 2016). Triticale typically has higher fiber due to its robust straw, which can reduce digestibility (Ferraretto et al., 2015). Barley entries (2020, 2021) showed improved fiber (lower ADF/NDF), likely increasing energy (Baleghn et al., 2022).

RFV results confirmed barley varieties outperformed triticale in most years. Lower fiber directly correlates with higher energy and improved animal performance (Baleghn et al., 2022). Barley (2021) achieved the highest energy due to favorable low fiber and balanced DM. Low RFV in triticale (2019) highlights the typical biomass-quality trade-off for this species (Leiebert et al., 2023).

Environmental and genetic contributions to silage quality trends

The observed increase in RFV and DM over time likely reflects both genetic improvements and better crop management. Higher RFV indicates greater energy availability for lactation and aligns with Loučka et al. (2015), who reported strong climatic effects on silage energy traits, especially in Central Europe.

The decline in NDF and ADF suggests improved digestibility and cell wall breakdown, likely due to better genotypes or optimized harvest timing. Similar genotype × year interactions for fiber traits have been noted in tropical grasses and sorghum (Singh et al., 2023). Crude protein remained stable, which is expected given its stronger genetic control and lower environmental sensitivity (Pedersen et al., 1983). Still, the stable protein alongside improved energy and fiber indicates balanced nutritional gains.

The slight pH drop in 2021 is notable, as lower pH supports better lactic acid fermentation and preservation consistent with findings in sweet sorghum and maize bagasse (Yucel et al., 2023).

Multivariate structure of silage traits and genotype year interactions

PCA revealed clear multidimensional patterns and trade-offs in silage quality. PC1, explaining most variance, showed a strong antagonism between fiber (ADF, NDF) and energy (RFV, DM) traits. This confirms high cell wall content negatively impacts digestibility and energy (Singh et al., 2023). RFV and DM vectors opposing ADF and NDF in the loading plot indicate energy-rich silage has lower structural fiber, typical for modern hybrids (Loučka et al., 2015).

Protein content, primarily on PC2, was not closely aligned with other traits. This suggests protein concentration is governed by factors distinct from fiber or energy, likely genotype-specific influences on nitrogen assimilation and proteolysis during ensiling (Pedersen et al., 1983). Its orthogonal nature in the biplot supports its independence as a selection criterion.

Temporal clustering in the PCA score plot showed 2021 genotypes mostly on PC1's negative side, consistent with higher RFV and DM that year. This suggests favorable environmental conditions or efficient genotype-by-environment (G×E) interactions in 2021, aligning with previous findings on climatic/soil impacts on forage quality (Zebeli and Metzler-Zebeli, 2012).

These multivariate patterns are informative for breeding and silage management. Genotypes on the negative PC1 axis (primarily 2021) are suitable for high-performance dairy due to higher energy density. Those on the positive PC1 axis (higher fiber) may suit dual-purpose systems where biomass is valued. PC1 represents nutritional efficiency (RFV, DM, fiber), while PC2 captures protein and fermentation-related variation (pH). These dimensions align with research on fiber accumulation and digestibility trade-offs (Jung and Allen, 1995; Oba and Allen, 1999). Genotypic dispersion along PC1 shows the feasibility of selecting forage genotypes for improved feeding value and adaptability to annual environmental fluctuations. PCA biplots offer a practical decision-support tool for breeders and agronomists, aiding informed selection strategies for specific nutritional and agronomic goals.

Implications of trait correlations for silage quality optimization

The correlation patterns among silage traits highlight key physiological trade-offs relevant to breeding and management. The strong negative relationship between fiber components (ADF, NDF) and energy traits (RFV, DM) reflects the well-known challenge of improving digestibility without compromising cell wall strength. Krüger et al. (2020) confirmed this in corn silage, linking high ADF/NDF to lower starch, TDN, and dry matter density critical factors in dairy energy intake.

The moderate positive DM–RFV correlation suggests DM captures both moisture and energy content, aligning with studies showing DM relates to carbohydrate accumulation and aerobic stability (Mukamuhirwa et al., 2024). Thus, DM optimization is not just for preservation but also essential for meeting nutritional needs in high-producing cows.

Crude protein showed weak, inconsistent ties to other traits, indicating regulation via distinct genetic or physiological pathways. Jayanegara et al. (2020) noted that while protein improves digestibility, it may raise pH and proteolysis, effects unrelated to fiber. Similarly, Wang et al. (2024) found that as soybean forage matured, protein increased while NDF and ADF declined, supporting their physiological separation.

pH was largely uncorrelated with other traits, reflecting its dependence on microbial activity and ensiling conditions rather than plant composition a finding consistent with Mukamuhirwa et al. (2024).

These results underscore the need for multivariate selection in breeding. Enhancing fiber digestibility without sacrificing DM or protein requires careful trait balancing and genomic tools. The independence of protein and fermentation traits further suggests that improving nutritional value demands integrated strategies that consider genotype-by-environment interactions and fermentation dynamics.

Conclusion

This study assessed silage quality traits of 20 hooded barley lines, four standard cultivars, and one triticale control under the agro-climatic conditions of the Harran Plain (Şanlıurfa, Turkey). Aimed at identifying superior forage types and supporting forageoriented breeding, the findings highlight the potential of hooded barley to improve regional roughage supply and ruminant nutrition.

Significant variation in traits such as pH, dry matter, crude protein, ADF, NDF, and RFV across genotypes and years reflected both genetic and environmental effects. Several hooded lines showed silage quality values comparable to or in some cases even higher than those of the standard barley cultivars and the triticale check, as supported by the statistical groupings. This confirms their promise as safe, efficient forage sources due to the absence of awns.

The identification of nutritionally superior hooded lines supports their use in local forage systems, helping to reduce Turkey's forage deficit, especially during periods of green feed shortage. PCA and correlation analyses clarified trait relationships, guiding targeted breeding strategies.

In summary, this research advocates for the adoption and development of silage-optimized hooded barley. The promising genotypes identified offer valuable resources for breeding programs aiming to boost forage quality; however, since yield-related traits were not assessed in this study, their effect on yield performance requires further investigation. Future studies should include multi-location trials, explore genetic drivers of silage performance, and assess the economic viability of large-scale cultivation for sustainable forage production in Turkey.

REFERENCES

[1] Adeboyejo, F. O., Olatunde, S. J., Rustria, G. A., Azotea, A. N. B., Ostonal, J. M., Reyes, M. J. A., Oyeyinka, S. A. (2023): Asian Fermented Cereal Based Products. – In: Indigenous Fermented Foods for the Tropics. Academic Press, San Diego, pp. 37-56. https://doi.org/10.1016/B978-0-323-98341-9.00002-5.

[2] AOAC (2005): Official Methods of Analysis. – 18th Ed. AOAC International, Gaithersburg, MD.

[3] Bai, R., Li, H., Chen, S., Yuan, X., Chen, Y., Huang, Y., Zhou, Q.-Y., Guan, H. (2024): Microbiome and response surface methodology analyses reveal Acetobacter pasteurianus as the core bacteria responsible for aerobic spoilage of corn silage (Zea mays L.) in hot and humid areas. – Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2024.1473238.

[4] Balehegn, M., Ayantunde, A., Amole, T., Njarui, D., Nkosi, B. D., Müller, F. L., Adesogan, A. T. (2022): Forage conservation in sub-Saharan Africa: review of experiences, challenges, and opportunities. – Agronomy Journal 114: 75-89. https://doi.org/10.1002/agj2.20954.

[5] Borreani, G., Tabacco, E., Schmidt, R. J., Holmes, B. J., Muck, R. A. (2018): Silage review: factors affecting dry matter and quality losses in silages. – Journal of Dairy Science 101: 3952-3979. https://doi.org/10.3168/jds.2017-13837.

[6] Cherney, J. H., Cherney, D. J. R. (2003): Assessing Silage Quality. – In: Buxton, D. R., Muck, R. E., Harrison, J. H. (eds.) Silage Science and Technology. ASA-CSSA-SSSA, Madison, WI, pp. 141-198. https://doi.org/10.2134/agronmonogr42.c4.

[7] Duniere, L., Sindou, J., Chaucheyras-Durand, F., Chevallier, I., Thévenot-Sergentet, D. (2013): Silage processing and strategies to prevent persistence of undesirable microorganisms. – Animal Feed Science and Technology 182(1-4): 1-15. https://doi.org/10.1016/j.anifeedsci.2013.04.006.

[8] Elakhdar, A., Solanki, S., Kubo, T., Abed, A., Elakhdar, I., Khedr, R., Qualset, C. O. (2022): Barley with improved drought tolerance: challenges and perspectives. – Environmental and Experimental Botany 201: 104965. https://doi.org/10.1016/j.envexpbot.2022.104965.

[9] Ferraretto, L. F., Shaver, R. D. (2015): Effects of whole-plant corn silage hybrid type on intake, digestion, ruminal fermentation, and lactation performance by dairy cows through a meta-analysis. – Journal of Dairy Science 98: 2662-2675. https://doi.org/10.3168/jds.2014-9045.

[10] Hansey, C. N., Lorenz, A. J., de Leon, N. (2010): Cell wall composition and ruminant digestibility of various maize tissues across development. – BioEnergy Research 3: 28-37. https://doi.org/10.1007/s12155-009-9068-4.

[11] Hoy, M. D., Moore, K. J., George, J. R., Brummer, E. C. (2002): Alfalfa yield and quality as influenced by establishment method. – Agronomy Journal 94: 65-71. https://doi.org/10.2134/agronj2002.6500.

[12] Huang, B., Huang, D., Hong, Z., Owie, S. O., Wu, W. (2020): Genetic analysis reveals four interacting loci underlying awn trait diversity in barley (Hordeum vulgare L.). – Scientific Reports 10: 12535. https://doi.org/10.1038/s41598-020-69335-x.

[13] Huang, B., Wu, W., Hong, Z. (2021): Genetic interactions of awnness genes in barley. – Genes 12: 606. https://doi.org/10.3390/genes12040606.

[14] Jatkauskas, J., Vrotniakiene, V., Eisner, I., Witt, K. L., do Amaral, R. C. (2024): Comparison of the chemical and microbial composition and aerobic stability of high moisture barley grain ensiled with either chemical or viable lactic acid bacteria application. – Fermentation 10: 62. https://doi.org/10.3390/fermentation10010062.

[15] Jayanegara, A., Wardiman, B., Kondo, M., Ridla, M., Laconi, E. B. (2020): Fermentative quality of silage as affected by protein level in the ensiled material: a meta analysis. – IOP Conference Series: Earth and Environmental Science 462: 012001. https://doi.org/10.1088/1755-1315/462/1/012001.

[16] Jung, H. G., Allen, M. S. (1995): Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. – Journal of Animal Science 73: 2774-2790. https://doi.org/10.2527/1995.7392774x.

[17] Kitaw, G., Terefe, G., Faji, M., Mengistu, G., Dejene, M., Fekadu, D., Mekonnen, B. (2024): Effect of maize (Zea mays L.) genotypes, harvesting stages and ensiling periods on fermentation and nutritional value of silage. – Grass Research 4(1). https://doi.org/10.48130/grares-0024-0006.

[18] Kleinschmit, D. H., Schmidt, R. J., Kung, L. Jr. (2005): The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. – Journal of Dairy Science 88: 2130-2139. https://doi.org/10.3168/jds.S0022-0302(05)72889-7.

[19] Krüger, A. M., Lima, P. D. M. T., Abdalla Filho, A. L., de Geus Moro, J., De Carvalho, I. Q., Abdalla, A. L., Jobim, C. C. (2020): Dry matter concentration and corn silage density: effects on forage quality. – Tropical Grasslands—Forrajes Tropicales 8: 20-27. https://doi.org/10.17138/TGFT(8)20-27.

[20] Liebert, J., Cherney, J. H., Ketterings, Q. M., Mirsky, S. B., Pelzer, C. J., Ryan, M. R. (2023): Winter cereal species, cultivar, and harvest timing affect trade offs between forage quality and yield. – Frontiers in Sustainable Food Systems 7: 1067506. https://doi.org/10.3389/fsufs.2023.1067506.

[21] Loučka, R., Hakl, J., Jirmanová, J., Tyrolová, Y. (2015): Yearly variation in maize silage fermentation and nutritive quality. – Grass and Forage Science 70: 674-681. https://doi.org/10.1111/gfs.12151.

[22] Makkar, H. P. S. (2018): Feed demand landscape and implications of food-not-feed strategy for food security and climate change. – Animal 12: 1744-1754. https://doi.org/10.1017/S175173111700324X.

[23] Marković, M., Markov, S., Pejin, D., Mojović, L., Vukašinović, M., Pejin, J., Joković, N. (2011): The possibility of lactic acid fermentation in the triticale stillage. – Chemical Industry and Chemical Engineering Quarterly 17: 153-162. https://doi.org/10.2298/CICEQ100916065M.

[24] MGM (2021): Monthly Meteorological Data of Şanlıurfa. – Turkish State Meteorological Service, Ankara. https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=&m=SANLIURFA.

[25] Muck, R. E. (2010): Silage microbiology and its control through additives. – Revista Brasileira de Zootecnia 39(Suppl. Esp.): 183-191. https://doi.org/10.1590/S1516-35982010001300021.

[26] Mukamuhirwa, M. L., Hatungimana, E., Latré, J., Ntawubizi, M. (2024): Piloting locally made microsilos to monitor the silage fermentation quality of conserved forage in Rwanda. – Research Square. https://doi.org/10.21203/rs-4339458/v1.

[27] Nair, J., Christensen, D., Yu, P., Beattie, A. D., McAllister, T., Damiran, D., McKinnon, J. J. (2016): A nutritional evaluation of common barley varieties grown for silage by beef and dairy producers in western Canada. – Canadian Journal of Animal Science 96: 598-608. https://doi.org/10.1139/cjas-2016-0032.

[28] Nikkhah, A. (2012): Barley grain for ruminants: a global treasure or tragedy. – Journal of Animal Science and Biotechnology 3: 22. https://doi.org/10.1186/2049-1891-3-22.

[29] Oba, M., Allen, M. S. (1999): Evaluation of the importance of digestion kinetics of fiber in forage and total mixed diets. – Journal of Dairy Science 82: 589-597. https://doi.org/10.3168/jds.S0022-0302(99)75271-9.

[30] Pedersen, J. F., Haskins, F. A., Gorz, H. J., Britton, R. (1983): Variability for traits used to estimate silage quality in forage sorghum hybrids. – Crop Science 23: 376-379. https://doi.org/10.2135/cropsci1983.0011183X002300020045x.

[31] Polan, C. E., Stieve, D. E., Garrett, J. L. (1998): Protein preservation and ruminal degradation of ensiled forage treated with heat, formic acid, ammonia, or microbial inoculant. – Journal of Dairy Science 81: 765-776. https://doi.org/10.3168/jds.S0022-0302(98)75633-4.

[32] Rohweder, D. A., Barnes, R. F., Jorgenson, N. (1978): Proposed hay grading standards based on laboratory analyses for evaluating quality. – Journal of Animal Science 47: 747-759. https://doi.org/10.2527/jas1978.473747x.

[33] Seydosoglu, S. (2019): Effects of different mixture ratios of grass pea (Lathyrus sativus L.) and barley (Hordeum vulgare) on quality of silage. – Legume Research—An International Journal 42(5): 666-670. https://doi.org/10.18805/LR-468.

[34] Singh, S., Singh, T., Singh, K. K., Srivastava, M. K., Das, M. M., Mahanta, S. K., Misra, A. K. (2023): Evaluation of global Cenchrus germplasm for key nutritional and silage quality traits. – Frontiers in Nutrition 9: 1094763. https://doi.org/10.3389/fnut.2022.1094763.

[35] Stewart, B. A., Lal, R. (2018): Increasing world average yields of cereal crops: it's all about water. – Advances in Agronomy 151: 1-44. https://doi.org/10.1016/bs.agron.2018.05.001.

[36] Tan, M., Yolcu, H. (2021): Current status of forage crops cultivation and strategies for the future in Turkey: a review. – Journal of Agricultural Sciences 27: 114-121. https://doi.org/10.15832/ankutbd.903732.

[37] Wang, K., Sun, S., Zou, Y., Gao, Y., Wang, B., Hua, Y., Lu, Y., Qin, L. (2024): Effect of growth stage on nutrition, fermentation quality, and microbial community of semidry silage from forage soybean. – Plants 13: 739. https://doi.org/10.3390/plants13050739.

[38] Xie, Y., Wang, L., Li, W., Xu, S., Bao, J., Deng, J., Hu, Z., Yu, Z. (2022): Fermentation quality, in vitro digestibility, and aerobic stability of total mixed ration silage in response to varying proportion alfalfa silage. – Animals 12(8): 1039. https://doi.org/10.3390/ani12081039.

[39] Yucel, C., Bilgin, F. D., İnal, İ., Hatipoğlu, R. (2023): Bagasse yield and quality traits of silage made from juice-extracted sweet sorghum (Sorghum bicolor var. saccharatum (L.) Mohlenbr.) stalks. – Emirates Journal of Food & Agriculture 35(4). https://doi.org/10.9755/ejfa.2023.v35.i4.3017

[40] Zebeli, Q., Metzler-Zebeli, B. U. (2012): Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle. – Research in Veterinary Science 93: 1099-1108. https://doi.org/10.1016/j.rvsc.2012.02.004.