

SILAGE CHARACTERISTICS OF MAIZE-FORAGE LEGUME MIXTURES ENSILED AT DIFFERENT HARVEST PERIODS

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ABSTRACT

This study was conducted to evaluate the effects of different harvest periods on the ensiling characteristics of maize-forage legume mixtures. Sole maize (*Zea mays* L.), sole common vetch (*Vicia sativa* L.), sole lablab (*Lablab purpureus*), maize + common vetch, and maize + lablab intercrops were harvested on different days after planting (DAP). The experiment was conducted using a completely randomized design with a 5 (forage systems) x 3 (harvest periods) factorial treatment arrangement and three replicates. Forage samples were ensiled at early, medium, and late harvest maturity: 50, 75 and 100 DAP. After ensiling in a laboratory mini-silo, samples were collected to determine chemical composition, microbial populations, and fermentation quality. Early harvest demonstrated lower contents of dry matter (DM) and crude protein, with values of 16.99 and 15.84% DM, respectively, in sole forage silages. High water-soluble carbohydrate concentrations and low fibre values ($p < 0.05$) were recorded when ensiled forage mixtures were harvested at the medium maturity stage. In mixed silages, the medium harvest time resulted in significantly lower populations of enterobacteria, yeasts and moulds, and higher populations of lactic acid bacteria, with values of 3.61, 4.83 and 5.17 $\log_{10}$ cfug⁻¹, respectively. Lower pH values, NH₃-N content, and greater lactic acid production ($p < 0.05$) were observed when the ensiled mixture and sole forages were harvested at the medium maturity stage compared to early and late harvest periods. Mixed silages from the medium harvest produced lower butyric acid and higher acetic and propionic acid concentrations (0.19, 1.07 and 1.25% DM, respectively) in the medium harvest, followed by silages from the late and early harvests. A medium harvest for ensiling mixed forage has the potential to produce high-quality silage for ruminant feeding.

Keywords: Mixed crop silage, harvest periods, nutrient composition, microbial populations, fermentation quality.

INTRODUCTION

Maize, forage legume lablab, and common vetches are among the most commonly cultivated forages by smallholder farmers in Southern Africa, including Zimbabwe and South Africa (Rahman et al., 2022). Maize (*Zea mays* L.) is the

most important cereal and annual crop grown in summer for three main purposes: human food, feed for poultry, and fodder for livestock (Massey et al., 2016). Lablab (*Lablab purpureus*) and common vetch (*Vicia sativa* L.) are forage legumes grown to provide supplementary winter forage (Mohammad, 2017). Improved productivity

of maize grain could increase food availability and dietary diversity for humans, as it contains essential macro- and micronutrients (Ullah et al., 2010). Moreover, productivity of lablab, common vetch, and maize forage could provide additional feed during periods of fodder shortage, thereby supporting livestock nutritional requirements.

Agronomic strategies that involve the growth of two or more crops, including forage legumes and cereals, such as maize crops on the same piece of land, lead to more efficient use of land and more available feed for people and livestock, in particular, dry season feeding during winter and spring months (Matusso et al., 2014). Forage legumes grown in intercropping systems with maize crops could be effective options for increasing forage production and providing fodder and grain for feeding livestock. This would also benefit smallholder farmers by reducing animal feed scarcity, improving supplementary feeding, and increasing livestock products, such as meat and milk, thereby contributing to improved food security and greater economic returns for local households (Komarek et al., 2015).

However, forage harvest and processing used to feed livestock, particularly silage making, pose potential challenges in smallholder farmers' systems (Weinberg, 2023). A major barrier preventing smallholders from using silage in livestock feeding in Lesotho is the lack of knowledge regarding key steps in the silage-making process, such as harvesting forage crops at an appropriate maturity stage, wilting to achieve optimal moisture levels, and properly chopping and compacting the material. Furthermore, another challenge in forage production is the harvest time that limits the yield and quality of fodder for livestock. Smallholder farmers mainly conserve forage in the form of hay; therefore, forage materials prepared for the hay-making process are usually harvested at the late maturity stage. Forage quality generally declines as plants mature; therefore, delayed harvesting at a late stage results in reduced forage quality. This leads to lower palatability, higher fibre content, and decreased energy and protein levels in ruminant feed, ultimately reducing livestock productivity.

Therefore, cultivation of maize and forage legumes in intercropping mixtures and harvested at the optimum maturity stage for silage making could mitigate carbohydrate, protein and other nutritional deficiencies during periods of fodder scarcity. Ensiling may serve as a practical method for preserving forage to feed livestock after harvest and during dry seasons. In fact, it has proven to be an efficient solution during periods of low forage availability, providing high-quality feed for ruminants (Pariz et al., 2017). Silage can

store large amounts of nutrients to meet livestock energy requirements (Ribeiro et al., 2017). Among the crops recommended for ensiling, maize has attracted interest, being widely used with positive results. The crop has good patterns of microbial fermentation and high contents of digestible starch, water-soluble carbohydrates (WSCs) and fibre (Merino et al., 2021). In addition, forage legumes have gained increasing importance for silage production due to their high annual production, ranging from 1 and 10 ton of dry matter (DM) per hectare (t/ha), and large harvesting windows (Serbester et al., 2015).

Careful selection of forage species and optimal harvest time can offer significant advantages for silage production. Zhang et al. (2017) reported that maize harvested at the flowering stage exhibited superior silage characteristics compared to other maturity stages, based on parameters such as pH, ammonia nitrogen, acetic acid, butyric acid and propionic acid. According to Guo et al. (2017), digestibility and energy and protein levels of ensiled forage crops were highest when cut during the early vegetative growth stage.

The legume harvest at the early bloom stage guarantees high-quality silage in terms of nutrient digestibility (Nadeau et al., 2010). Forages present great chemical and nutritional quality, easy mechanical harvesting and high protein value, creating a high protein feed suitable for ruminants when cut before the flowering stage (Bernardes et al., 2018). Marchesini et al. (2019) found that after 60 days of silage, DM content, WSC content, and crude protein (CP) yield in maize decreased at the late vegetative growth stage, while neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents tended to increase. The inclusion of forage legumes during the growth of maize and grasses helps to balance protein and energy levels and may have potential effects on ensiling. However, forage legumes alone have low contents of sugars and high buffering capacity, which leads to difficulties in preservation (Kumar, 2019). In this study, it was hypothesized that maize-forage legume mixtures may not exhibit significantly different silage characteristics when ensiled at varying harvesting periods. Therefore, the present study aimed to evaluate the effects of different harvest periods on the ensiling characteristics of maize-forage legume mixtures.

MATERIALS AND METHODS

Site Description

A field experiment was conducted at St. Michael, Lesotho (coordinates: 29° 25' 38 S, 27° 40' 50 E, and altitude 1700 m above sea level). Maize (*Z. mays* L.) was intercropped with common vetch

(*V. sativa* L.) or lablab (*L. purpureus*) under rainfed conditions for two years (2021-2022). The site lies in a lowland region of Lesotho, characterized by warm summers and cold winters. Annual rainfall ranges from 500 mm to 1000 mm, with the highest values in the summer season (Lesotho Meteorological Services, 2023). Winters are generally dry and cold and are characterized by dry air and warm to moderate temperatures during the day, with a sudden cold temperature just after sunset. The average annual temperature ranges between 15.2 and 25.6 °C in the lowland region. The predominant soils of the area are classified as fersiallitic soils with an orthic A horizon (Fey, 2010).

Agronomic practices

Maize, lablab, and common vetch were grown as sole crops or as maize with a legume cover crop in 10 × 5 m plots replicated three times. A basal fertilizer (6:3:4 NPK) was applied to all maize plots at a rate of 75 kg ha⁻¹ at sowing. Legume-only plots received a combination of P and K at 80 kg ha⁻¹. Planting spacing for sole maize was 75 cm between rows and 30 cm between plants, while for sole common vetch and lablab, spacing was 40 cm between rows and 10 cm between plants. Lablab and common vetch were intercropped between two maize rows at 37.5 cm apart from the maize row with inter-row and 10 cm intra-row spacing. The plant density of maize was 40,000 plants ha⁻¹, while the forage legumes (lablab and common vetch) were planted at 60,000 plants ha⁻¹. All necessary agronomic practices, including weed, pest, and disease control, were uniformly applied across all experimental plots during the cropping season. For silage preparation, a one-meter-long row from each plot was harvested at ground level. Maize and forage legume samples were harvested at 50, 75, and 100 days after planting (DAP), and the chemical composition of forage samples was assessed immediately prior to ensiling.

Silage preparation in forage systems

The harvested forage materials were wilted overnight to reduce moisture content to 50 – 45%. Maize and legume materials were chopped to lengths of approximately 3 – 4 cm using a forage cutter. Silage was prepared from sole maize (MZ), sole common vetch (CV), sole lablab (LB), and in mixtures of maize + common vetch (MZ + CV), and maize + lablab (MZ + LB) from the intercrops. Maize + common vetch (MZ + CV) and maize + lablab (MZ + LB) planting density combinations were 40/60 thousand plants ha⁻¹. The maize-to-forage ratio was 1:1 during the preparation of maize-legume silages. Forage samples were

ensiled at three harvest periods: early (50 DAP), medium (75 DAP), and late (100 DAP). Molasses, diluted with distilled water, was evenly sprayed over the layer of chopped maize-forage legume intercrops and thoroughly mixed to ensure uniform distribution. Molasses was applied at a rate of 8.6 g kg⁻¹ fresh forage along with distilled water (1.4 g kg⁻¹). Forage materials were ensiled in laboratory mini-silos consisting of a 1-litre polyethylene bottle with a capacity of 2.0±0.2 kg. After filling, the mini-silos were compacted using a pressing apparatus to expel as much air as possible to ensure sustainable anaerobic conditions. The experiment was conducted using a completely randomized design with a 5 (forage systems) × 3 (harvest periods) factorial treatment arrangement and three replicates. The mini-silos were incubated at room temperature (±25 – 30°C) for 60 days.

Silage chemical composition and fermentation characteristics

The silages were open after 60 days. Samples were collected and freeze-dried using an Alpha 1-4 LSC freeze drier (Martin Christ Freeze Dryer, Germany) at -60 °C and at a pressure of 0.011 mbar for 24 h. Freeze-dried samples were ground using a laboratory mill (Thomas Wiley model 4, Arthur Thomas & Co.) with a 1 mm screen, and subsequently stored for chemical analysis. CP was determined using AOAC (2016) procedures; NDF and ADF contents were determined following the method of Van Soest et al. (1991), while *in vitro* dry matter digestibility (IVDMD) was assessed using the protocol described by Tilley and Terry (1963).

Microbial populations and silage pH were measured on the day of silo opening. Immediately after opening the mini-silos, 40 grams of each fresh silage from each sample was weighed and transferred to a sealable container containing 350 mL of distilled water. The samples were subjected to different serial dilutions using distilled water, beginning with an initial dilution followed by subsequent dilutions to ensure thorough homogenization, as described by Kavanagh (1947). The containers were sealed and shaken for 6 h at 180 rpm using a horizontal shaker. Following agitation, silage extracts were filtered through four layers of cheesecloth for further analysis.

An electrode pH meter in a water-based solution was used to measure pH. The plate culture method was used to determine microbial populations of the fresh silages. Twenty grams of each sample was weighed and shaken well with 100 mL of sterilized distilled water and diluted in a sodium chloride solution (0.90%). Lactic

acid bacteria (LAB) were counted on an agar plate of Lactobacilli MRS broth after incubation in an anaerobic box at 40°C for at least 24 h. Enterobacteria counts were determined on a plate of violet red bile agar with lactose after anaerobic incubation at 35°C for 24 h (Weber et al., 2009). Yeasts and moulds were counted on potato dextrose agar plates after incubation at 30°C for 24 h (Liu et al., 2002). For analysis of the microbial population, colonies were counted, and their numbers were expressed as viable numbers of microorganisms in colony forming units per gram of fresh matter (cfug⁻¹) and transformed into log₁₀ of cfug⁻¹ (Chang et al., 1995).

For the analysis of lactic acid and volatile fatty acids (VFAs), silage extracts (2 mL) were filtered and acidified with meta-phosphoric acid (25%) to reduce the pH. The acidified samples were then centrifuged for 15 min using a capillary column at 40°C to determine lactic acid and VFAs, including acetic acid, butyric acid, and propionic acid, following the method of Khorvash et al. (2006). To determine silage ammonia nitrogen (NH₃-N), 25 mL of silage extract was homogenized in a stomacher blender for 3 min and filtered through Whatman filter paper (Filya, 2002). Total fatty acids (TFAs) were determined using AOAC (2016) procedures, and WSC content was measured by colorimetry following reaction with anthrone reagent, as outlined by McDonald et al. (1991).

Statistical analysis

Analyses of variance were performed using the general linear model (GLM) procedures of the Statistical Analysis System (SAS, 2023). The sources of variation included harvesting period, forage type, and their interaction, as specified in the model. Dependent variables included nutrient composition, microbial populations, and fermentation products. Treatment means were compared using a the protected least significant difference (LSD) at a significance level of $p < 0.05$. The model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ijk}$$

Where Y_{ijk} is the variable response; μ is the general mean; α_i is the harvesting period effect; β_j is the forage type effect; $\alpha\beta_{ij}$ is the harvesting period by forage type interaction; and ϵ_{ijk} is the residual error.

RESULTS

Fresh forage and ensiled forage chemical composition

The chemical composition of fresh forages before ensiling is presented in Table 1. It was

observed that the chemical composition was significantly ($p < 0.05$) variable among different harvest times. The mean DM, CP, NDF and ADF values of different harvest times ranged from 25.28 to 28.91, 12.97 to 13.74, 35.81 to 39.85, and 35.14 to 41.94% DM respectively. Lower DM, CP, NDF and ADF concentrations were found in forages harvested at the early maturity stage compared to medium and late stages. Chemical composition was affected significantly ($p < 0.05$) by the forage systems treatments. The mean DM, CP, NDF and ADF contents for the forage systems were in the range of 17.45 – 37.16, 6.73 – 24.23, 31.05 – 44.94, and 27.65 – 47.41% DM, respectively. Sole forages had lower DM and ADF contents, and higher CP and NDF values than the mixtures.

Forage composition indices varied significantly ($p < 0.05$) due to the different harvest times. The IVDMD, WSC, and TFA values across harvest times ranged from 53.55 to 56.94, 9.26 to 10.15, and 2.40 to 3.02% DM respectively. Forages harvested at medium harvest time showed higher ($p < 0.05$) IVDMD, WSC, and TFA values as compared to those harvest at late and early maturity stages. Among the different forage systems, IVDMD, WSC, and TFA concentrations varied from 50.82 to 58.66, 6.95 to 11.66, and 1.16 to 4.47% DM, respectively, with the lowest values observed in sole-cropped forages. A significant interaction ($p < 0.05$) between forage systems and harvest time was observed for chemical composition parameters. Specifically, medium harvest time resulted in higher values of CP concentration ($p < 0.05$) in ensiled sole forage legumes and greater IVDMD, WSC, and TFA contents in ensiled forage mixtures compared to the other maturity stages evaluated.

The chemical composition of the ensiled forage is presented in Table 2. Different harvest times had a significant effect ($p < 0.05$) on the chemical composition of the silages. DM and CP contents varied from 23.41 to 27.04 and 12.90 to 13.67% DM, respectively. The late harvest time showed higher DM content as compared to medium and early harvests. The highest CP content ($p < 0.05$) was found in medium harvest followed by late and early harvests. Forage systems had significant effects ($p < 0.05$) on chemical composition. The DM and CP contents across forage systems ranged from 15.58 to 35.29 and 6.65 to 24.16% DM, respectively. DM content ($p < 0.05$) was higher in mixed silages than in sole forage silages. Lower CP content ($p < 0.05$) was obtained in mixed silages compared to sole common vetch and lablab silages. The interaction of different harvest times and forage systems had significant effects ($p < 0.05$) on the contents of DM and CP. Early harvest resulted in lower DM and

Table 1. Chemical composition of fresh forage harvested at different maturity stages before ensiling.

Forage systems ¹	Harvest time ²	DM ³	CP (% DM)	NDF (% DM)	ADF (% DM)	IVDMD (% DM)	WSC (% DM)	TFA (% DM)
MZ	Early	20.42i	6.21f	37.31d	34.02e	44.66k	10.44c	2.83c
	Medium	27.17g	7.12f	42.97c	42.31d	55.07h	11.44ab	3.70bc
	Late	31.31f	6.85f	49.27a	47.33b	52.73j	10.81bc	3.36bc
CV	Early	16.41k	23.40b	35.45f	23.40i	53.84i	6.55f	0.85d
	Medium	17.86j	24.69a	36.29ef	28.29h	55.38gh	7.36e	1.24d
	Late	18.07j	24.59a	37.21de	31.27fg	55.05h	6.93ef	1.40d
LB	Early	19.77i	18.13d	42.95c	30.81g	54.76h	7.57e	1.14d
	Medium	21.58h	19.11c	45.47b	32.04f	56.48ef	8.39d	1.43d
	Late	22.17h	18.97cd	46.41b	33.29e	56.31fg	8.01de	1.59d
MZ+CV	Early	33.86e	8.33e	30.31j	41.86d	57.10def	11.31ab	3.38bc
	Medium	34.85d	8.68e	31.03i	46.53c	58.18bc	12.12a	3.78b
	Late	35.06cd	8.62e	31.81i	48.80a	57.96cd	11.55ab	3.67bc
MZ+LB	Early	35.95c	8.78e	33.03h	45.62c	57.41cde	10.44c	3.81b
	Medium	37.61b	9.11e	33.87gh	47.60b	59.57a	11.44ab	4.94a
	Late	37.93a	9.02e	34.54g	49.02a	58.99ab	10.81bc	4.67a
SEM ⁴ (±)		1.12	1.05	0.97	1.17	0.65	0.13	0.18

^{a-k} Values with different letters within the same column are significantly different ($P < 0.05$).

¹ Forage systems are Maize (MZ), Common vetch (CV), Lablab (LB), Maize+Common vetch (MZ+CV), and Maize+Lablab (MZ+LB).

² Harvest times correspond to: early for 50 days after planting; medium for 75 days after planting; and late for 100 days after planting.

³ DM: Dry matter, CP: Crude protein, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, IVDMD: *In vitro* dry matter digestibility, WSC: water soluble carbohydrates, and TFA: Total fatty acid.

⁴ SEM: Standard error of mean.

CP contents in sole forage silages compared to those observed in the medium and late harvests.

It was observed that NDF and ADF contents were significantly variable ($p < 0.05$) among different harvest times. NDF and ADF concentrations for different harvest times were in the range of 33.78 – 37.82 and 33.17 – 39.97% DM, respectively. The early harvest showed lower NDF content ($p < 0.05$) as compared to the medium and late harvests. The lowest ADF concentration ($p < 0.05$) was observed in the early harvest followed by the medium and late harvests. NDF and ADF values varied significantly ($p < 0.05$) due to different forage systems. Across different forage types, NDF and ADF contents were 29.02 to 42.91 and 25.68 to 45.44% DM, respectively. Higher NDF concentration ($p < 0.05$) was observed in sole forage silages compared to mixed silages. ADF content was lower in sole forage silages compared to that in mixed silages. The interaction of different harvest times and forage systems had significant effects ($p < 0.05$) on the concentration of NDF and ADF. The late harvest showed a higher concentration of NDF and ADF in sole maize and mixed silages with respect to the medium and early harvests.

Different harvest times had a significant effect ($p < 0.05$) on forage composition indices. IVDMD, WSC, and TFA values ranged from 55.38 to 58.77, 9.57 to 10.51 and 3.03 to 3.65% DM, respectively, across different harvest times. The medium harvest exhibited higher IVDMD, WSC and TFA values ($p < 0.05$) than the late and early harvests. Across forage systems, IVDMD, WSC and TFA values varied from 52.65 to 60.48, 7.03 to 12.37 and 1.79 to 5.09% DM, respectively. Sole forage silages had lower IVDMD, WSC and TFA values ($p < 0.05$) than mixed silages. Different harvest times and forage systems showed significant effects on IVDMD, WSC and TFA for fermented forages. Higher IVDMD, WSC and TFA values ($p < 0.05$) for mixed silages were recorded in the medium harvest period, while intermediate and low values were observed in the late and early harvest times, respectively. A similar trend was also observed in sole forage silages across harvest times.

Ensiled forage microbial populations

Microbial population counts in the ensiled forage are presented in Table 3. Microbial composition recorded a significant variation

Table 2. Effects of three harvest times on the chemical composition of ensiled forage.

Forage systems ¹	Harvest time ²	DM ³	CP (% DM)	NDF (% DM)	ADF (% DM)	IVDMD (% DM)	WSC (% DM)	TFA (% DM)
MZ	Early	18.53j	6.13k	35.27d	32.05h	46.49m	10.52g	3.46e
	Medium	25.30g	7.05i	40.94c	40.34f	56.90i	11.52de	4.33c
	Late	29.43f	6.78j	47.24a	45.36c	54.56l	10.88f	3.99d
CV	Early	14.54m	23.33b	33.42e	21.43n	55.67k	6.63l	1.48i
	Medium	15.99l	24.62a	34.26de	26.32m	57.21h	7.44j	1.87gh
	Late	16.20l	24.52a	35.18d	29.30k	56.88i	7.01k	2.03fg
LB	Early	17.90k	18.06e	40.92c	28.84l	56.59j	7.65j	1.77h
	Medium	19.71i	19.03c	43.44b	30.07j	58.31g	8.47h	2.06fg
	Late	20.30h	18.90d	44.38b	31.32i	58.14g	8.08i	2.22f
MZ+CV	Early	31.99e	8.26h	28.28h	39.89g	58.93f	11.37e	4.01d
	Medium	32.98d	8.61g	29.00gh	44.56d	60.01c	12.19c	4.41c
	Late	33.19d	8.55g	29.78g	46.83a	59.79d	11.63d	4.30c
MZ+LB	Early	34.08c	8.71g	31.00f	43.65e	59.24e	11.67d	4.44c
	Medium	35.73b	9.04f	31.84f	45.63b	61.40a	12.92a	5.57a
	Late	36.06a	8.95f	32.51ef	47.05a	60.82b	12.51b	5.30b
SEM ⁴ (±)		1.18	1.03	0.89	1.26	0.52	0.11	0.21

^{a-n} Values with different letters within the same column are significantly different ($P < 0.05$).

¹ Forage systems are Maize (MZ), Common vetch (CV), Lablab (LB), Maize+Common vetch (MZ+CV), and Maize+Lablab (MZ+LB).

² Harvest times correspond to: early for 50 days after planting; medium for 75 days after planting; and late for 100 days after planting.

³ DM: Dry matter, CP: Crude protein, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, IVDMD: *In vitro* dry matter digestibility, WSC: water soluble carbohydrates, and TFA: Total fatty acid.

⁴ SEM: Standard error of mean.

($p < 0.05$) among the different harvest times. Populations of LAB, enterobacteria, and yeasts and moulds varied from 4.97 to 5.07, 3.93 to 4.04 and 4.97 to 5.08 $\log_{10}$ cfug⁻¹, respectively, across the different harvest periods. LAB from fermented forages harvested at the medium maturity stage was higher ($p < 0.05$) than that of the late and early maturity stages. In addition, fermented forages harvested at the medium harvest time exhibited lower ($p < 0.05$) enterobacteria populations, while the lowest number of yeast and mould populations were found in fermented forages harvested at the medium maturity stage compared to other maturity stages.

Microbial composition was affected significantly ($p < 0.05$) by the forage systems treatments. Populations of LAB, enterobacteria, and yeasts and mould ranged from 4.38 to 5.43, 3.62 to 4.81 and 4.86 to 5.28 $\log_{10}$ cfug⁻¹, respectively, across forage systems. Sole forage silages resulted in lower populations of LAB than mixed silages. Conversely, enterobacteria, and yeast and mould counts were lower ($p < 0.05$) in mixed silages. The interaction forage systems $\times$ different harvest times had a statistically significant effect ($p < 0.05$) on all the evaluated microorganisms. The

medium harvest time resulted in significantly higher population of LAB ($p < 0.05$), and lower populations of enterobacteria, yeasts and mould in mixed silages, followed by the late and early maturity stages.

Fermentation profile

The fermentation products of ensiled forage are presented in Fig. 1. Across harvest times, pH values ranged from 3.64 to 3.96; NH₃-N X from 5.84 to 8.26% total nitrogen TN; and lactic acid production from 6.62 to 10.02% DM across different harvest times. Fermented forage harvested at the medium maturity stage had lower pH values and NH₃-N content, and higher lactic acid production ($p < 0.05$) than the early and late maturity stages. Across forage systems, pH values ranged from 3.21 to 4.22; NH₃-N from 3.61 to 13.99% TN; and lactic acid production from 5.92 to 11.88% DM, respectively. Mixture silages had lower pH values and NH₃-N content ($p < 0.05$) compared to sole silages such as sole maize, sole lablab, and sole common vetch. Lactic acid production was higher ($p < 0.05$) in sole silages than in mixture silages. Different harvest times and forage systems showed a significant effect (p

Table 3. Effects of three harvest times on microbial composition of ensiled forage.

Forage systems ¹	Harvest time ²	LAB ³	ENT ³	Y&M ³
		Count of microorganisms (log ₁₀ cfug ⁻¹)		
MZ	Early	4.35k	4.88a	5.34a
	Medium	4.41j	4.75c	5.21c
	Late	4.39j	4.81b	5.29b
CV	Early	4.95i	3.98d	5.12d
	Medium	5.04g	3.85e	5.03f
	Late	5.01h	3.94de	5.06f
LB	Early	5.32c	3.91e	5.09e
	Medium	5.55a	3.85ef	4.96h
	Late	5.41b	3.86ef	5.01fg
MZ+CV	Early	5.09f	3.76g	4.94h
	Medium	5.15e	3.66h	4.85i
	Late	5.13e	3.74g	4.86i
MZ+LB	Early	5.13e	3.67h	4.92h
	Medium	5.18d	3.55i	4.81j
	Late	5.15e	3.65h	4.84i
SEM ⁴ (±)		0.09	0.06	0.07

^{a-k} Values with different letters within the same column are significantly different ($P < 0.05$).

¹ Forage systems are Maize (MZ), Common vetch (CV), Lablab (LB), Maize+Common vetch (MZ+CV), and Maize+Lablab (MZ+LB).

² Harvest times correspond to: early for 50 days after planting; medium for 75 days after planting; and late for 100 days after planting.

³ LAB: Lactic acid bacteria, ENT: Enterobacteria and Y&M: Yeasts and moulds.

⁴ SEM: Standard error of mean.

< 0.05) on pH, $\text{NH}_3\text{-N}$ and lactic acid for forage silages. Lower pH values, $\text{NH}_3\text{-N}$ content, and greater lactic acid production ($p < 0.05$) were observed when the ensiled mixture and sole forages were harvested at the medium maturity stage compared to the early and late maturity stages.

The acetic, propionic, and butyric acids of different harvest times were in the range of 1.14 to 1.45, 0.54 to 0.75, and 0.15 to 0.25% DM, respectively. Lower acetic and propionic acid and the highest butyric acid concentrations ($p < 0.05$) were found in ensiled forage harvested at the early maturity stage compared to medium and late maturity stages. Concentrations of acetic, propionic, and butyric acid ranged from 0.94 to 1.74, 0.07 to 1.25, and 0.02 to 0.39% DM, respectively, across forage systems. Mixed silages have higher acetic and propionic acid and lower butyric acid concentrations ($p < 0.05$) than sole forage silages. The forage systems $\times$ different harvest times interaction for acetic, propionic, and butyric acid concentrations were statistically significant ($p < 0.05$). The medium harvest time resulted in significantly ($p < 0.05$) lower butyric acid and higher acetic and propionic acid in

mixed silages, followed by the late and early maturity stages.

DISCUSSION

Nutrient composition

During the dry season, ensiled forage crops have potential as a source of feed for livestock. Harvesting forage at different maturity stages and ensiled might result in distinct silage characteristics. Therefore, it is important to identify a suitable maturity stage to harvest forage for ensiling. It is well known that DM and CP are important indicators reflecting the quality characteristics of silage (Zhu et al., 2022). In this study, late harvest time had higher DM than that of medium and early harvest times. This might be attributed to an increase in dry-matter accumulation throughout the growth period. Guo et al. (2019) also reported that DM content was lower at an earlier growth stage compared to late stages.

The sole forage silages had lower DM content compared with silage mixtures. Bengisu (2019) also noted the difference in DM content between sole and mixture silages. CP levels are a direct

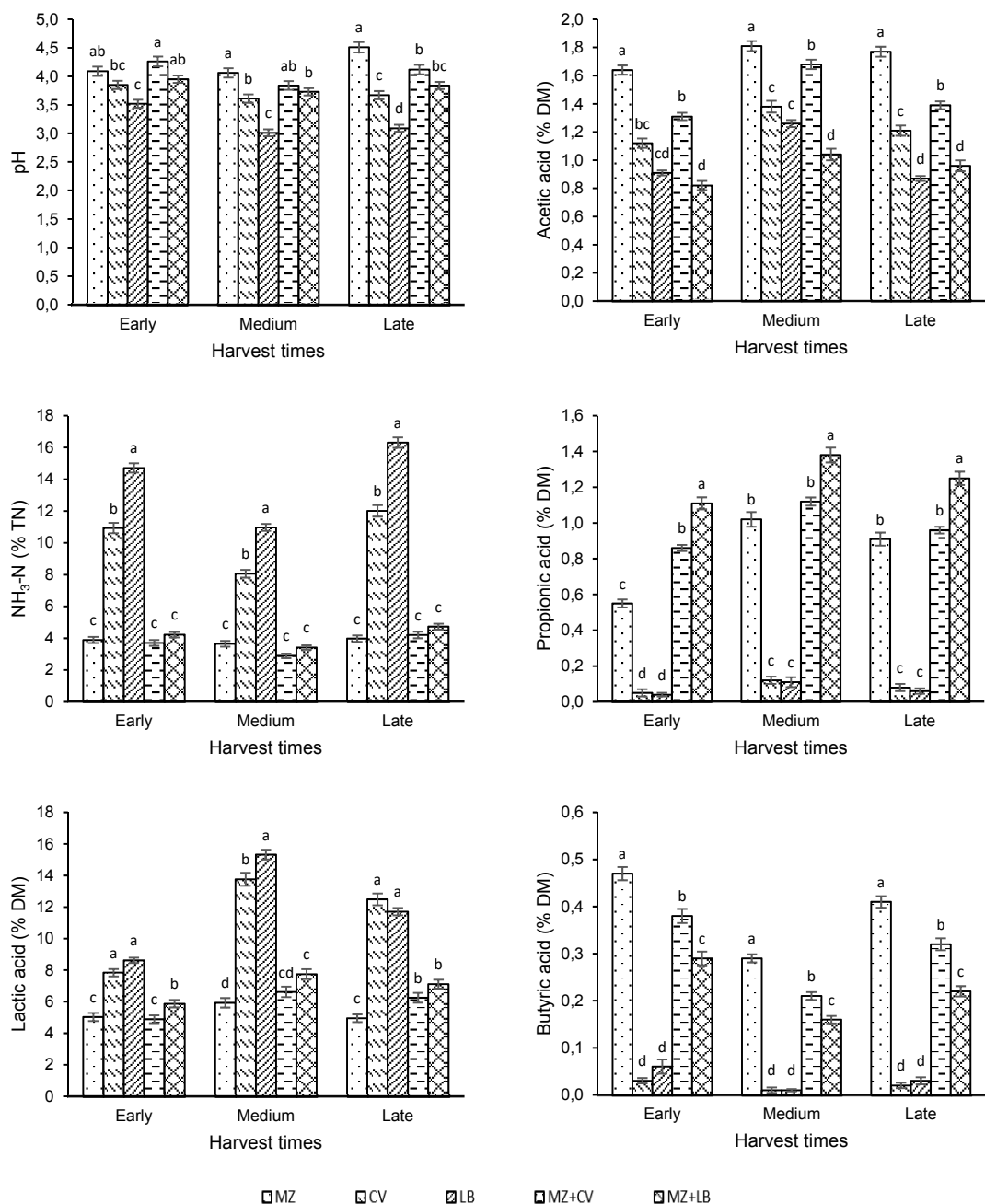


Figure 1. Effects of three harvest times on the fermentation products of ensiled forage.
^{a-d} Values with different letters within the bar charts are significantly different ($P < 0.05$). The error bar represents the standard error of the mean (SEM). Harvest times correspond to: early for 50 days after planting, medium for 75 days after planting; and late for 100 days after planting. Forage systems are Maize (MZ), Common vetch (CV), Lablab (LB), Maize+Common vetch (MZ+CV), and Maize+Lablab (MZ+LB).

reflection of the quality of the forage at the time of harvest (Salama et al., 2020). In the present study, CP contents were higher when forage was harvested at the medium maturity stage compared to the other maturity stages. This may be associated with the high IVDMD content

observed at that stage. This agrees with Marković et al. (2022) who reported that CP levels are influenced by the stage of maturity at harvest. Higher CP content was observed in ensiled sole forage legumes compared to mixtures, which is likely to be explained by the presence of high

buffering capacity in legumes. Previous studies reported similar effects when ensiling legumes with cereal mixtures (Özyazici et al., 2022).

It is well documented that NDF and ADF are commonly used to analyse forages, as they represent total fibre content (Xu et al., 2023). In the present study, ensiled forage harvested at early stage had reduced NDF and ADF contents compared with the other harvest times. These results are consistent with the findings of Topcu et al. (2021), who reported differences in NDF and ADF across different harvest times. Furthermore, NDF content was lower in mixed silages than that in sole forage silages, probably due to reduced accumulation of structural components in mixtures at harvest, possibly associated with lignification process (Hendricks et al., 2020). In general, legume inclusion in mixtures resulted in lower total NDF contents due to greater lignification as compared to cereal crops (Yang et al., 2023). Moreover, the mixtures were harvested and ensiled within a relatively narrow range of maturity. Extremes in NDF concentrations may occur when ensiled at an over-mature stage (Cherney et al., 2020). The lowest ADF content was obtained in sole silages compared to mixed silages, which conceides with the results of Ashoori et al. (2021).

IVDMD, WSC, and TFA concentrations are key parameters to assess silage quality (Nascimento-Ramos et al., 2019). In the present study, these indicators varied across the different harvest times. Ensiled forage harvested at the medium maturity stage produced higher concentrations of IVDMD, WSC, and TFA compared to late and early maturity stages. This may be attributed to an adequate supply of fermentable carbohydrates, which contributed to the production of high-quality silage (Dong et al., 2022a). These results are consistent with those described by Dong et al. (2022b) when exploring the chemical composition of fermented forage cut at different maturity stages. Additionally, IVDMD, WSC, and TFA concentrations were lower in sole silages compared to mixed silages, consistent with the results of Tao et al. (2020) when ensiling legume-maize mixtures. In the present study, the interaction of different harvest times and forage systems had significant effects on the nutritional composition. This may be attributed to agronomics practices, particularly harvesting forage at stages with higher contents of CP, OM, and IVDMD, along with lower levels of NDF and ADF. This agrees with Salama (2019), who reported that harvest time has a significant impact on the nutritional content of forage materials.

Silage microbial composition

Microbial composition, including LAB, enterobacteria, and yeasts and moulds, plays a significant role in the fermentation process and influences silage quality (Wang et al., 2022). In the present study, LAB formation was lower in sole silages compared to mixed silages, whereas enterobacteria, yeasts and mould counts tended to be lower in the mixed silages. The lower WSC content obtained in sole legume silages likely failed to support high LAB populations. Di Miceli et al. (2023) reported a similar effect when ensiling legumes with cereals. Additionally, higher LAB counts were observed in ensiled forage at the medium harvest time. However, lower populations of enterobacteria, yeasts and moulds were observed at the same harvest time, likely due to higher LAB counts and lower pH values. Similar results were reported by Wang et al. (2021) when exploring the microbial community and fermentation characteristics of ensiled forage mixtures. In the present study, the interaction between harvest time and forage system had significant effects on microbial composition. This may be attributed to the high accumulation of WSC in forage mixtures at harvest, which provided sufficient fermentable sugars for LAB and supported a desirable fermentation process (Gonda et al., 2023).

Silage fermentation indices

Fermentation products like pH, $\text{NH}_3\text{-N}$, and lactic acid affect the ensiling process and demonstrate whether an excellent, average, or poor fermentation has occurred (Zhao et al., 2021). In the present study, the late harvest time had higher pH, increased $\text{NH}_3\text{-N}$ content, and lower lactic acid production compared to the other treatments. This may be attributed to the restricted fermentation resulting from late sampling time relative to harvest, which is associated with dry forage materials that are overly mature (Okoye et al., 2023). Moreover, pH values and $\text{NH}_3\text{-N}$ content were higher in the sole silages than in mixtures, whereas lactic acid production was lower in sole silages compared to mixed silages. This is likely to be explained by the low WSC content produced in sole forages at the time of harvest. Forage crops ensiled with low fermentable carbohydrates lead to silage with higher pH values and ammonia nitrogen content, and lower acetic and propionic acid concentrations (Chen et al., 2019). These results are similar to those reported by Gülümser et al. (2021) for ensiled forage legumes with cereal mixtures.

Organic acids such as acetic, propionic, and butyric acid have long been used to assess silage

quality and inhibit the development of spoilage microorganisms during the fermentation process (Hisham et al., 2022). In the present study, the concentration of acetic and propionic acid was higher (and that of butyric acid lower) in the medium harvest time compared to the other harvest periods. The lower concentrations of acetic and propionic acids may be attributed to an unrestricted ensiling process facilitated by low pH values (Zhao et al., 2022). These findings are consistent with those of Terler et al. (2021), when ensiling forage harvested at different sampling periods.

Furthermore, low acetic, higher butyric, and decreased propionic acid concentrations were produced in the sole silages compared with mixture silages. High concentrations of butyric acid have been associated with low energy value in silage, leading to lower feed intake and, in some cases, ketosis in lactating cow, ultimately compromising production (Steinbrenner et al., 2019). Furthermore, silage with high concentrations of butyric acid and ammonia nitrogen, along with low levels of acetic acid and propionic acids, often contains significant amounts of other undesirable end products, such as amines, which may negatively affect animal performance (Jia et al., 2021). Zhang et al. (2023) reported a similar effect when soybeans were ensiled alone or combined with maize. In the present study, the interaction of different harvest times and forage systems showed significant effects on the fermentation characteristics of silage. This is likely to be attributed to adequate fermentable carbohydrates at harvest time and might have produced the metabolism of hetero-fermentative LAB, leading to desired pH values in well-preserved silages (Weinberg, 2023). Fermentable carbohydrates were mainly consumed by LAB to produce lactic acid, resulting in low $\text{NH}_3\text{-N}$ levels and reduced pH values during the fermentation process (Wang et al., 2022). Silage with low pH values, reduced $\text{NH}_3\text{-N}$ contents, and low butyric acid concentrations suggests that the mixed forage was well preserved during ensiling (Ahmed et al., 2023).

CONCLUSIONS

The chemical composition of ensiled forage harvested at the medium maturity stage was superior to that at early and late maturity stages. Sole forage silages exhibited a lower nutritional value compared to mixed silages. The results showed that forage legumes can be planted as cover crops with maize, cut at medium sampling time relative to harvest, and ensiled to

maintain the nutrient composition required for livestock feeding. The medium harvest period resulted in improved microbial composition with lower populations of enterobacteria, yeasts and moulds, and greater populations of LAB compared to the early and late harvest periods. Sole forage silage had lower LAB counts, greater enterobacteria populations, and higher yeast and mould counts than mixed silage. The results suggest that fermented forage mixtures harvested in the medium maturity stage may exhibit enhanced microbial composition and improved silage quality for livestock feeding. Improved fermentation products were obtained in ensiled forage harvested at the medium stage compared to early and late maturity stages. Furthermore, fermentation quality was found to be higher in mixed silages compared to sole silages. The results revealed that harvest at the medium stage improves the fermentation characteristics of mixed silages. Moreover, these improved characteristics make mixed silages a valuable feed option, particularly during periods of pasture scarcity such as the dry season.

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Author contributions

Poloko Mosebi: Active participation in the development of the methodology; Casper Madakadze: Review of the final version of the article; Moeketsi Ntakatsane: Active participation in the discussion of the results; Ratsele Ratsele: Active participation in the bibliographic review. All authors approved the final version of the manuscript.

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