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Indigenous plant resources as potential probiotic sources for fermentative feed enhancements: a semi-arid perspective

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ABSTRACT
This study investigates the potential of indigenous plant resources as probiotic enhancers for improving fermentative feed in Lupane District, a rural semi-arid region in Zimbabwe facing significant livestock production challenges due to limited feed availability and environmental stressors. The objective was to identify, characterize, and assess the feasibility of using indigenous plants to enhance maize silage through probiotic activity. A mixed-method approach was adopted, combining a field survey to identify available indigenous plant species and an experimental trial to evaluate their probiotic effects. The survey identified several locally available plants, but due to their utility and availability, *Colophospermum mopane*, *Moringa oleifera*, *Aloe barbadensis* Miller, *Vachellia karroo, and Terminalia sericea* (leaves and seeds) were selected for further investigation. Microbiological characterization followed, with Gram staining revealing the presence of *Lactobacillus* spp., *Bacillus* spp., and *Enterococcus* spp. in *Colophospermum mopane* and *Aloe barbadensis* Miller, while *Vachellia karroo* also showed notable microbial activity. For the experimental setup, inoculation was performed by adding naturally occurring microorganisms from the selected plants, without additional commercial inoculants. To assess the effects on silage quality, a completely randomized design was implemented, featuring seven treatments: maize mixed with each of the identified plant species at a ratio of 80% maize to 20% plant material, and a control consisting of 100% maize silage. Molasses (at a 1:2 ratio with water) was added to each silage mix at a rate of 5% per 4 kg of 2–3 cm cut maize to promote fermentation. The results demonstrated that the maize-*Colophospermum mopane* and maize-*Aloe barbadensis* Miller combinations outperformed the other silages across various parameters. Significant improvements ($p < 0.05$) were observed in nutrient composition, particularly in crude protein, fiber, and dry matter content, accompanied by a decrease in pH levels post-ensiling. However, no significant differences were noted in organoleptic properties, such as color, smell, texture, and mold occurrence, among the treatments. Nutritional analysis confirmed that *Colophospermum mopane* and *Aloe barbadensis* Miller possess strong potential to improve fermentation quality and nutrient retention in silage. These findings suggest that incorporating indigenous plants into silage production can enhance the nutritional value and overall quality of feed.

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Keywords: Additives, Fermentation, Indigenous Plants, life on land, Lupane District, Probiotics, Zimbabwe

RÉSUMÉ

Cette étude explore le potentiel des ressources végétales indigènes en tant qu'améliorateurs probiotiques
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pour les aliments fermentés dans le district de Lupane, une région rurale semi-aride du Zimbabwe confrontée à des défis importants dans la production de bétail en raison de la disponibilité limitée de fourrage et des stress environnementaux. L'objectif était d'identifier, de caractériser et d'évaluer la faisabilité de l'utilisation de plantes indigènes pour améliorer l'ensilage de maïs grâce à une activité probiotique. Une approche méthodologique mixte a été adoptée, combinant une enquête de terrain pour identifier les espèces végétales indigènes disponibles et un essai expérimental pour évaluer leurs effets probiotiques. L'enquête a identifié plusieurs plantes disponibles localement, mais en raison de leur utilité et de leur disponibilité, *Colophospermum mopane, Moringa oleifera, Aloe barbadensis Miller, Vachellia karroo et Terminalia sericea* (feuilles et graines) ont été sélectionnées pour une investigation plus approfondie. La caractérisation microbiologique a révélé, par coloration de Gram, la présence de Lactobacillus spp., Bacillus spp. et Enterococcus spp. dans Colophospermum mopane et Aloe barbadensis Miller, tandis que Vachellia karroo a également montré une activité microbienne notable. Pour l'expérience, l'inoculation a été réalisée en ajoutant des micro-organismes naturellement présents dans les plantes sélectionnées, sans inoculants commerciaux supplémentaires. Pour évaluer les effets sur la qualité de l'ensilage, un plan complètement aléatoire a été mis en œuvre, comprenant sept traitements : mélange de maïs avec chaque plante identifiée dans un rapport de 80 % de maïs à 20 % de matière végétale, et un témoin constitué de 100 % d'ensilage de maïs. De la mélasse (dans un rapport 1:2 avec de l'eau) a été ajoutée à chaque mélange d'ensilage à un taux de 5 % par 4 kg de maïs coupé en morceaux de 2–3 cm pour favoriser la fermentation. Les résultats ont montré que les combinaisons de maïs-*Colophospermum mopane et* maïs-*Aloe barbadensis* Miller ont surpassé les autres ensilages sur divers paramètres. Des améliorations significatives ($p < 0,05$) ont été observées dans la composition nutritionnelle, notamment en matière de protéines brutes, de fibres et de matière sèche, ainsi que dans les niveaux de pH après ensilage. Cependant, aucune différence significative n'a été notée dans les propriétés organoleptiques, telles que la couleur, l'odeur, la texture et la présence de moisissures, entre les traitements. L'analyse nutritionnelle a confirmé que Colophospermum mopane et Aloe barbadensis Miller possèdent un fort potentiel pour améliorer la qualité de la fermentation et la rétention des nutriments dans l'ensilage. Ces résultats suggèrent que l'intégration de plantes indigènes dans la production d'ensilage peut améliorer la valeur nutritionnelle et la qualité globale des aliments.

Mots clés : Additives, Fermentation, Indigenous Plants, life on land, Lupane District, Probiotics, Zimbabwe

INTRODUCTION

Globally, in semi-arid regions, livestock production faces on-going challenges due to limited access to high-quality feed and the compounded effects of environmental stressors like drought and extreme heat (Henry *et al*., 2018). This situation has been the norm for many rural districts, ultimately requiring innovative strategies to improve feed quality and animal nutrition while ensuring the sustainability of livestock systems. One such strategy is the use of indigenous plant resources as potential sources of probiotics to enhance the fermentative feed processes, offering a practical solution to mitigate the impact of environmental limitations on livestock nutrition (Gupta *et al*., 2022).

Indigenous plants, which have adapted to the harsh conditions of semi-arid ecosystems, play an essential role in local cultures and economies, providing food, medicine, fuel, and fodder (Boerma *et al*., 2004; Hadish, 2018). In semi-arid regions, these plants often possess unique nutritional profiles and probiotic properties that can enhance the quality of animal feed. However, despite their long-standing ecological and socio-economic importance, the potential of these plants as sources of probiotics for improving fermented feed remains largely untapped.

Ishawu (*Colophospermum mopane*) is a versatile tree with various uses, including food, medicine, and craftsmanship (Moyo *et al.*, 2020). *Inhlaba* (*Aloe barbadensis* Miller) is renowned for its medicinal properties, particularly in treating skin conditions and wounds (Mupfiga *et al.*, 2020). *Mufushwa* (*Moringa oleifera*) is a nutrient-rich plant with various health benefits, making it a popular choice for food and medicine (Okeniyi *et al.*, 2018). *Isinga* (*Vachellia karroo*) has cultural significance and is used in traditional ceremonies, while its leaves and pods have medicinal properties (Mavi *et al.*, 2019). *Mususu* (*Terminalia sericea*) is valued for its antimicrobial properties and is used to treat various ailments (Olajide *et al.*, 2019). These plants are deeply rooted in the local culture and are frequently mentioned due to their versatility, effectiveness, and cultural significance.

Probiotics, defined as live microorganisms that provide health benefits when consumed in sufficient quantities, are vital in fermentative processes such as ensiling (Aragon, 2012; Mugoti *et al.*, 2022; Ndudzo *et al.*, 2023). They enhance the nutritional value and preservation of silage by promoting beneficial microbial growth and inhibiting harmful pathogens (Vieco-Saiz *et al.*, 2019). Leveraging indigenous plant-derived probiotics could offer a sustainable, locally adapted solution to improve livestock feed quality, promote animal health, and build resilience against environmental stresses in semi-arid areas. This study aims to bridge traditional knowledge and modern scientific approaches to livestock nutrition. The research seeks to uncover the diversity, effectiveness, and the practical applications of indigenous plant-derived probiotics in semi-arid livestock systems, addressing the growing challenges faced in these environments.

MATERIALS and METHODS

This study was implemented as per approval by the Department of Animal Science at Lupane State University. Permission to carry out a survey in Lupane District was sought from the District Administration. The study was undertaken between November 2023 and May 2024 in the Lupane district of Matabeleland North Province of Zimbabwe. Lupane is found at a latitude of -19.4833º and longitude of 28.5500º. It covers an area of about 2500km² with a population of around 200 000 people. Lupane District is approximately 150km from Bulawayo, the second largest city of Zimbabwe, and is predominantly rural with most of the population engaged in subsistence farming. The vegetation is mostly semi-arid savanna, with a mixture of grasslands, shrubs and trees. The most dominant woody vegetation species in the area *are Terminaria sericia, Colophospermum mopane, Vachellia species and Combretum species*, which are well adapted to the dry climate (Mukungurutse *et al.*, 2018). The indigenous ethnic groups found in Lupane district are Ndebele and Tonga.

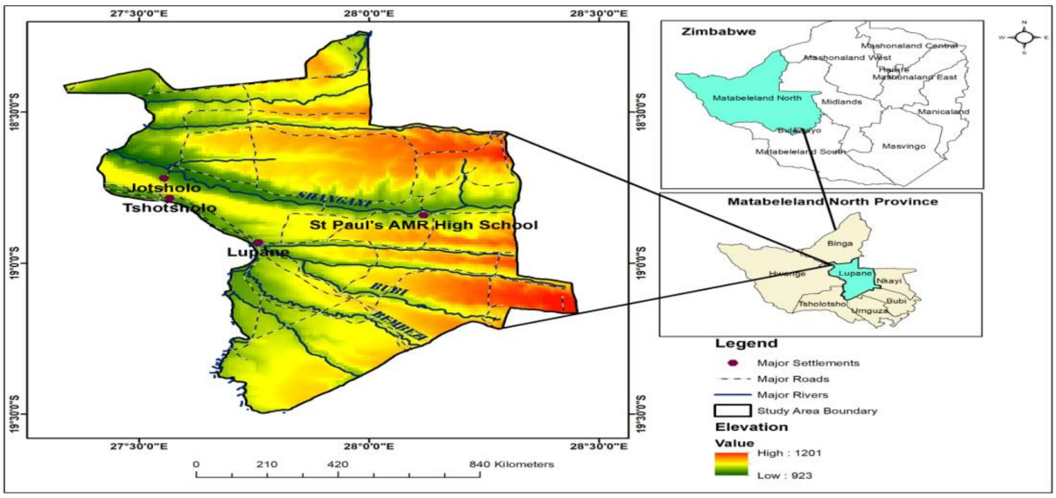


Figure 1. Study area (Source: QGIS).

Sampling procedures. Purposive sampling was used in selecting participants for the interviews. Sixty participants from 10 different wards of the Lupane district were selected based on their knowledge of indigenous plants and their experience with using probiotic products. Semi-structured questionnaires comprising open ended and closed ended questions were administered to the respondents face to face. The aim was to obtain names of different indigenous plant species, their uses and availability in the district.

Experimental design and layout. The study followed a completely randomized design (CRD) with no blocking factor. The layout was 7 treatments including a control with 3 replicates for each treatment. The treatments included *Colophospermum Mopane* leaves, *Moringa Oleifera* leaves, *Vachellia Karroo* leaves, *Aloe Barbadensis* Miller leaves, *Terminalia sericea* leaves and *Terminalia Sericea* seeds. The treatments: maize ratio was 1:9 respectively with the control having 100% maize only.

Maize production. Maize crop variety ZN 45 Zimbabwe super seeds were planted on a one-hectare piece of land at Lupane State University demo plot and left to grow under drip irrigation due to scarcity of rainfall to sustain their growth in semi-arid conditions. All management practices like weeding, fertilization, and pest control measures were followed until the crops reached the milky dough stage (Johansson, 2010). At this stage, the maize was harvested to ensure optimal nutritional quality (Johansson, 2010). Delaying harvesting allows the stover to become more lignified, and the over-mature kernels become harder and less digestible if left unbroken during ensiling (Buckerfield and Wilkins, 2001; McDonald *et al.*, 2018). The maize was randomly harvested across the plot by cutting at 10-12cm above the ground level.

Plant collection and identification of probiotics. From the plants which were identified in a survey, the leaves and seeds were collected and taken to the Lupane State University microbiology lab for microbiological identification of probiotics. Leaves were collected from *Moringa oleifera*, *Aloe barbadensis* Miller, *Colophospermum mopane*, *Vachellia karoo*, *Terminalia sericea* and seeds from *Terminalia sericea*,were put in Khakhi sample collection bags and transported to the laboratory in a cooler box.

Preparation of culture media. The collected plant materials were dried in an oven and ground to increase the surface area and release endophytic probiotics. They were weighed at 1.5g per sample and replicated 3 times to increase the precision of the results. Nutrient agar was prepared by dissolving 12.4g of nutrient agar powder in 400 mL of distilled water in a conical flask and then autoclaved at 121ºC for 20 minutes to sterilize it. It was allowed to cool to around 50ºC before it was poured into sterile- clean petri dishes at about 20ml per petri dish. The agar was then allowed to solidify at room temperature. Once solidified, the inoculants were sprinkled into the mixture with nutrient agar and incubated at 37ºC for 72 hours to allow optimal growth of bacteria. After 72 hours, bacteria of different shapes, sizes and forms were observed.

Gram staining. To accurately identify and classify bacterial cultures, a meticulous gram staining procedure was employed. Using inoculating loops, bacteria were carefully extracted from the media and smeared onto a pristine microscope slide. The bacteria were then heat-fixed to ensure adherence, followed by the application of crystal violet stain, the primary stain, which was allowed to set for one minute (O'Toole, 2016). After this initial staining, the slide was gently flooded with water to remove any excess stain. The next crucial step involved adding iodine for one minute, a process that effectively fixes the dye and enhances its retention (Tripathi *et al.*, 2023). Following the iodine treatment, excess iodine was washed away, and a brief exposure to ethanol, a decolourizer was applied for 2-5 seconds, being careful not to overexpose, as this could strip the stain from both gram-positive and gram-negative bacteria (Popescu, 1996). The final step involved the addition of safranin red, which was also rinsed off after one minute. Once dried, the slides were examined under a microscope, and the results were meticulously recorded in a table, ensuring a comprehensive analysis of the bacterial morphology and classification.

Silage preparation. The maize crop was chopped into 2-3 cm pieces using a machete and then placed into silage bags for the ensiling process. This study focused on seven distinct types of silages: maize alone, maize combined with *Aloe barbadensis* Miller, maize with *Colophospermum mopane*, maize and *Moringa oleifera*, maize paired with *Vachellia karoo*, maize mixed with *Terminalia sericea* leaves, and maize with *Terminalia sericea* seeds. Each treatment consisted of a precise ratio of 3.6 kg of forage to 0.4 kg of additives, with the control group consisting solely of 100% maize. To enhance fermentation, 5% molasses was incorporated at a ratio of 1 part molasses to 2 parts water (Mugoti *et al*., 2023) across all silage types. The fodder was carefully packed into the silage bags at 4 kg per sample, ensuring a gentle yet firm fill to eliminate any air pockets. Three bags were prepared for each silage type, with the forages densely packed to prevent air entrapment. Each bag was securely sealed with elastic strings and stored at room temperature to facilitate anaerobic fermentation over eight weeks (Azim *et al*., 1999). After this fermentation period, samples from each silage bag were collected for organoleptic evaluation and chemical analysis, paving the way for a comprehensive assessment of the silage quality.

Chemical analysis. The pre-dried samples underwent a comprehensive analysis to determine their nutritional composition, focusing on dry matter content, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and ether extracts (EE). For dry matter (DM) determination, samples were oven-dried at 65°C for 72 hours until they reached a constant weight. Ash content was assessed by incinerating samples in a muffle furnace at 600°C until they too achieved a constant weight, allowing for subsequent analysis of individual minerals. The Kjeldahl method was employed to quantify crude protein (CP); this involved digesting the samples in hot concentrated sulfuric acid, which liberated nitrogen as ammonia. Neutral detergent fibre (NDF) was evaluated by boiling the forage in a neutral detergent solution and measuring the resulting insoluble residue, which represents the cell wall contents. For acid detergent fibre (ADF) determination, samples were digested in an acid detergent solution, dissolving hemicellulose and leaving behind cellulose and lignin. Finally, ether extracts (EE) were quantified by passing hot petroleum ether (45^0C) through the feed sample to dissolve the crude fat, followed by evaporation of the solvent, leaving behind the ether extracts. This meticulous approach ensured accurate and reliable results for the nutritional analysis of the samples.

Physical analysis of silage. Upon opening the silage bags, a series of physical tests were conducted to evaluate the quality of all silages using a scoring system. From each replicate, two 500g samples were carefully extracted. One sample was designated for organoleptic assessments, focusing on key qualities such as smell, colour, and texture (Randa *et al*., 2017), as well as pH analysis utilizing a precise pH meter. The second sample underwent a thorough proximate analysis to determine essential nutritional components, including Dry Matter (DM), ash, ether extract (EE), crude protein (CP), Neutral Detergent Fiber (NDF), and Acid Detergent Fiber (ADF) (AOAC, 1990, 2016). Additionally, the average mold presence was recorded at the time of silage opening, and a standardized scale was employed to evaluate the organoleptic quality of the silage. This comprehensive analysis ensured a robust understanding of the silage's overall quality and nutritional value.

Table 1. Physical assessment of silages

	Scale				
Parameters	**0**	**1**	**2**	**3**	**4**
Color	Dark brown	Brown	Olive yellow	Light green	Olive green
Smell	Rancid butter smell	Sour vinegar smell	Sweet, fruity alcoholic aroma	Slightly sweet aroma	Sour milk aroma
Texture	Dry and course	Slightly dry and course	Moderately dry and course	Moist and course	Very moist and course

Data analysis. Data from the human-based survey on indigenous plants were meticulously entered into SPSS version 26.0 for comprehensive data analysis. This analysis aimed to ascertain the frequency of plant species mentioned by respondents. The findings were organized into a table, clearly displaying the frequency of each plant species alongside the percentage of respondents who identified them. To enhance understanding, a bar graph was generated using the chart builder feature in SPSS, visually illustrating the distribution of plant species, with plant names on the x-axis and their corresponding frequencies on the y-axis. The top five most frequently mentioned plants were selected for further experimentation.

Additionally, data regarding the presence and classification of probiotic strains were systematically collected and presented in a tabular format. This dataset included the names of plant species, bacterial isolates, gram-staining results, cell morphology, and colony characteristics. The chemical composition data underwent the Shapiro-Wilk test to assess normality before being analyzed using GenStat version 13 software. This analysis aimed to evaluate the potential of different plant species in enhancing silage quality. Descriptive statistics were employed to calculate mean values and standard deviations for various silage quality parameters. Furthermore, an Analysis of Variance (ANOVA) was conducted to identify significant differences in silage quality parameters across different plant species, with plant species included as a fixed effect in the model. To ensure clarity in the results, means were subjected to the Bonferroni test for separation, providing a robust statistical framework for interpreting the data.

RESULTS

Demographics

The demographic analysis (Figures 2-4) of the participants revealed a gender distribution of 45% male and 55% female. The age group was predominantly between 36 and 55 years old, comprising 75% of the sample. Education levels varied, with 58% of participants having completed primary education and 25% having secondary education. Experience with livestock was widespread, with over 50% of participants having more than 10 years of experience. Knowledge of indigenous plants was moderate to high in 70% of the participants. The knowledge of the use of probiotic products was reported by 1% of the sample. The results indicated a significant association between these two variables ($\chi^2 = 18.43$, $p < 0.001$). *Cramer's V*, a measure of the strength of association, was calculated to be 0.397, suggesting a moderate effect size. The frequencies of the different indigenous species are shown in Figure 5.

From the survey (Figure 5**)**, *Colophospermum mopane* was the most frequently mentioned indigenous plant with 10 out of 60 (16.7%) respondents mentioning it. This suggests that it is the most well-known or widely used plant among respondents. *Moringa oleifera* is the second most frequent with 7 (11.7%) mentions, indicating its popularity and potential use in livestock diets. *Aloe barbandesis* Miller is mentioned by 5 (8.3%) respondents, showing its moderate frequency and potential importance. *Terminalia sericea* (Ts) and *Vachellia karroo* were mentioned by 4 (6.7%) respondents each, indicating their relatively lower frequency compared to the top three plants.

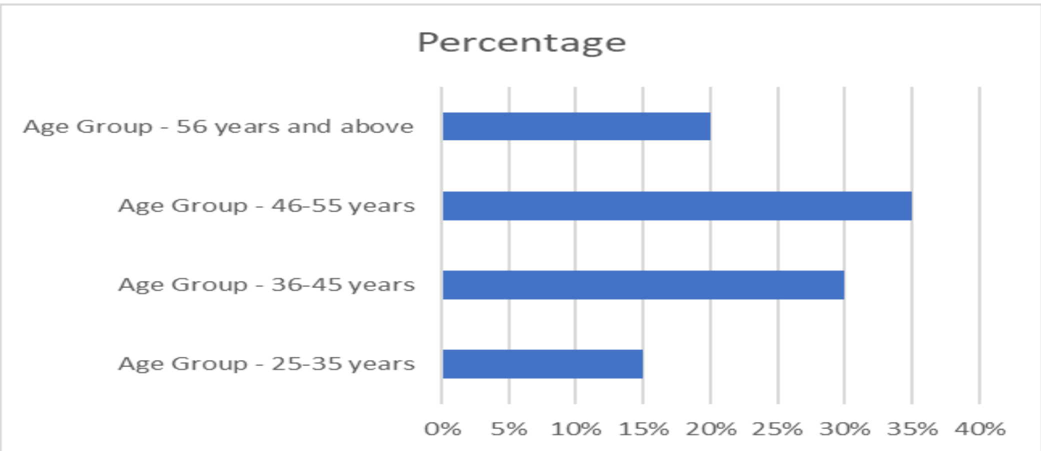
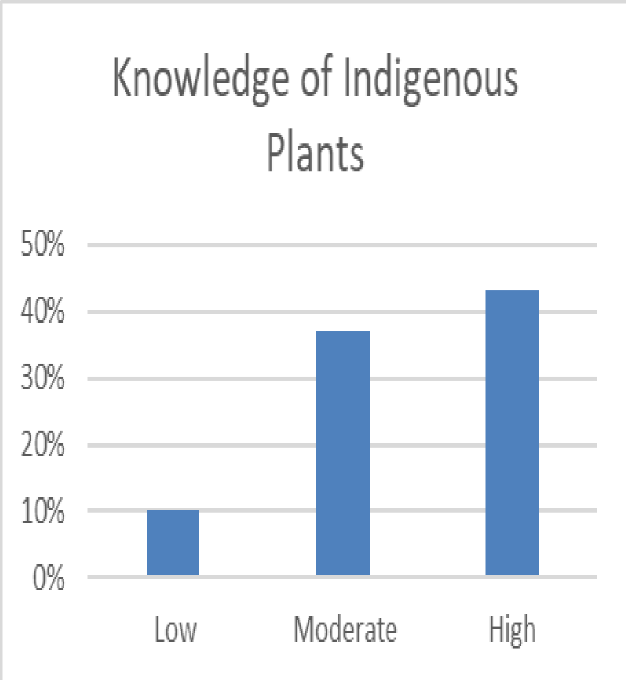


Figure 2. Age group by percentage



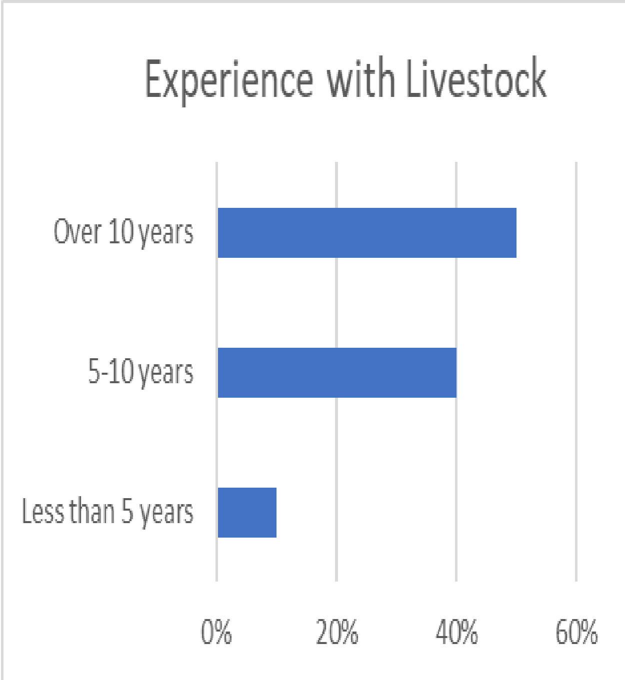


Figure 3. Knowledge of Indigenous plants

Figure 4. Experience with livestock

Table 2. Themes and Responses from the study

Theme	Response
Knowledge of Indigenous Plants	"We are familiar with plants like *Colophospermum mopane*, *Moringa oleifera*, *Aloe barbadensis miller*, and *Vachellia karroo*. These plants are common in our area."
Uses of Indigenous Plants	"We use *Moringa oleifera* for both human and livestock nutrition. *Colophospermum mopane* leaves are fed to goats, especially during the dry season."
Availability of Indigenous Plants	"The availability of plants like *Terminalia sericea* depends on the season. During the rainy season, it's easy to find, but in the dry months, it becomes scarce."
Experience with Probiotic Products	"We have heard of probiotics in commercial feeds, but we have not extensively used them."
Perceived Benefits of Using Plants	"We believe plants like *Aloe barbadensis* could help our livestock, especially during dry spells. If they improve digestion and feed quality, that would benefit our animals. Some even help with treating our livestock"
Challenges in Using Indigenous Plants	"We know these plants are useful, but we don't have the equipment or knowledge to properly prepare them for use as feed probiotics."
Local Practices for Livestock Feeding	"We already mix plants like *Mopane* leaves with other feeds during the dry season. It would be interesting to see if they could help fermentation as well."
Interest in Using Indigenous Plants	"If we can learn how to use our local plants to improve the quality of feed through fermentation, we would definitely try it. It would save us money on commercial feeds."

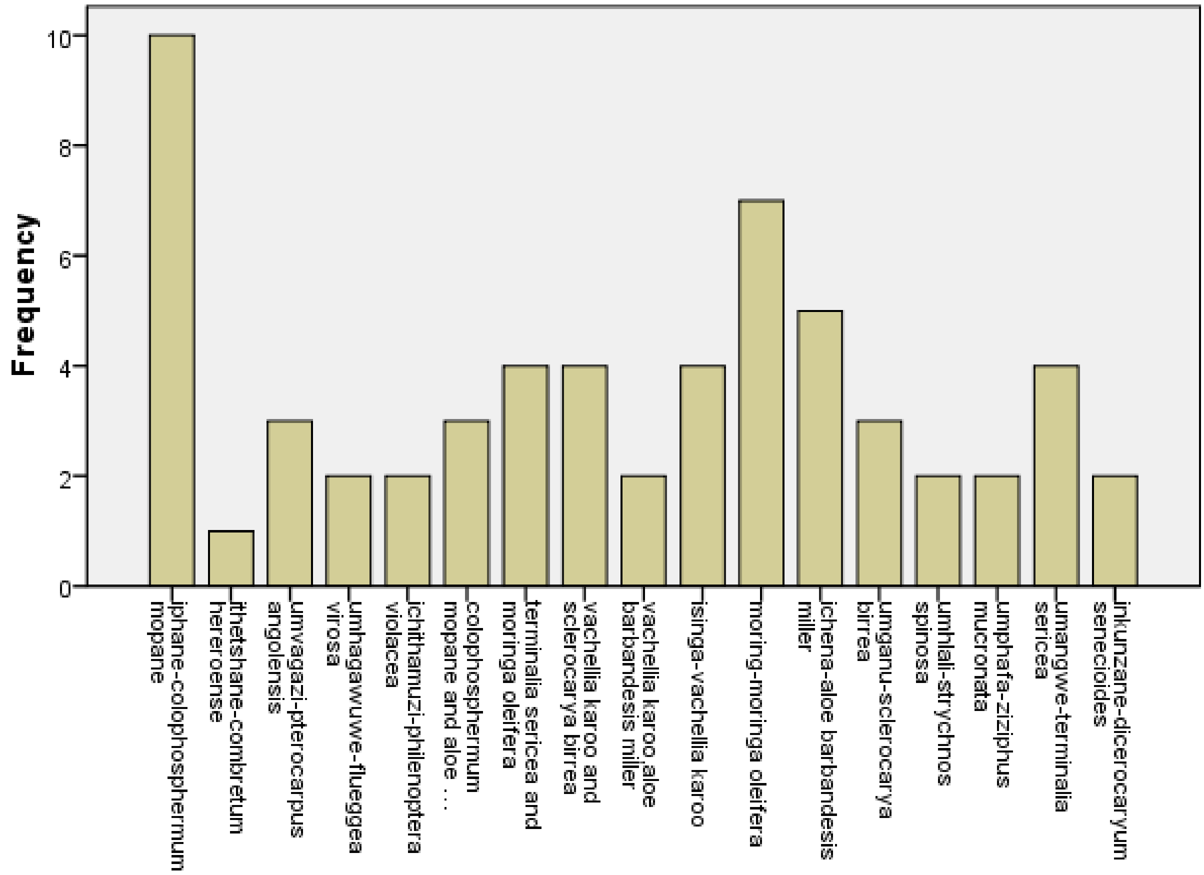


Figure 5. Indigenous plant frequencies

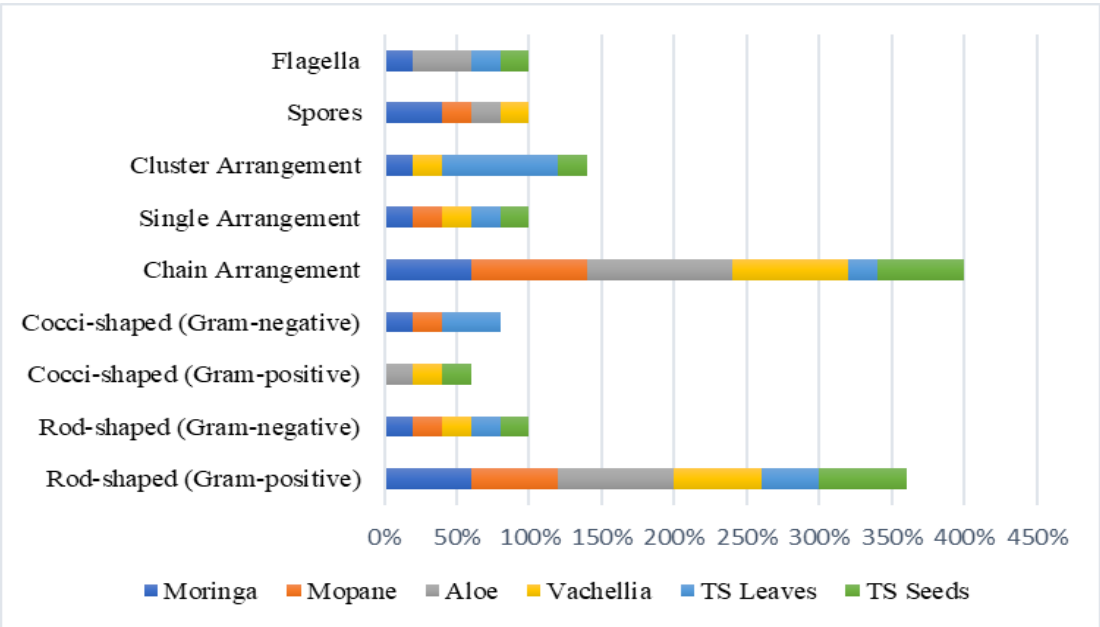


Figure 6. Comparison of bacterial morphology in plant samples

Table 3. Characterization of probiotics strains

Plant species	Bacterial isolate	Gram-staining result	Shape	Arrangement	Special colony characteristics
Moringa	B1	+	Rod	Chain	Spores
	B2	-	Rod	Single	-
	B3	-	Cocci	Cluster	-
	B4	+	Rod	Chain	-
	B5	+	Rod	Chain	Flagella
	B6	+	Cocci	Cluster	-
Mopane	B1	-	Cocci	Single	-
	B2	+	Rod	Chain	-
	B3	+	Rod	Chain	-
	B4	+	Rod	Chain	Spores
	B5	-	Rod	Single	-
	B6	-	Cocci	Cluster	-
Aloe	B1	+	Cocci	Chain	Flagella
	B2	+	Rod	Chain	Flagella
	B3	+	Rod	Chain	-
	B4	+	Rod	Chain	-
	B5	+	Rod	Chain	Spores
	B6	+	Rod	Chain	-
Vachellia	B1	+	Rod	Chain	-
	B2	+	Rod	Single	-
	B3	-	Cocci	Single	-
	B4	+	Cocci	Cluster	-
	B5	+	Rod	Chain	-
	B6	+	Rod	Chain	Spores
TS leaves	B1	-	Cocci	Cluster	-
	B2	-	Cocci	Single	-
	B3	+	Rod	Single	-
	B4	+	Rod	Cluster	-
	B5	+	Rod	Cluster	-
	B6	+	Rod	Chain	Flagella
TS Seeds	B1	+	Rod	Single	-
	B2	-	Cocci	Cluster	-
	B3	+	Cocci	Chain	-
	B4	+	Rod	Chain	-
	B5	-	Rod	Chain	-
	B6	+	Rod	Cluster	flagella

Plant name

Table 4. Ensiling Sensory Properties of Plant Samples Following Various Treatments

Parameter	Control	Moringa	*C. mopane*	Aloe	Vachellia	TS leaves	TS seeds
Color	Light green	Olive green	Olive green	Light green	Light green	Olive green	Light green
Smell	Moderately sour	Slightly sour	Moderately sour	Slightly sour	Moderately sour	Moderately sour	Moderately sour
Texture	Moderately moist and coarse	Moderately moist and coarse	Moderately moist and coarse	Moderately moist and coarse	Moderately moist and coarse	Moderately moist and coarse	Moderately moist and coarse

Table 5. Chemical analysis of silage

Variate	Treatments							p-value	LSD	Significance
	100% control	Moringa	C. mopane	Aloe	Vachellia	TS leaves	TS seeds			
DM	47.73^{b}	52.30^{c}	55.72^{d}	52.07^{c}	44.99^{a}	54.80^{d}	48.67^{b}	<.001	1.101	*
ASH	9.247^{e}	7.640^{b}	5.820^{a}	8.237^{c}	12.333^{f}	9.333^{e}	8.600^{d}	<.001	0.160	**
EE	1.343^{ab}	2.237^{d}	2.927^{e}	8.240^{f}	1.233^{a}	1.883^{c}	1.377^{b}	<.001	0.066	***
ADF	41.83^{f}	34.94^{c}	28.88^{a}	34.95^{c}	38.90^{e}	36.77^{d}	34.43^{b}	<.001	0.274	***
NDF	61.69^{f}	55.72^{c}	55.02^{b}	56.75^{d}	58.50^{e}	51.97^{a}	58.50^{e}	<.001	0.236	***
CP	8.527^{c}	8.627^{c}	8.707^{c}	8.160^{b}	6.617^{a}	8.533^{c}	8.983^{d}	<.001	0.116	***
pH	5.560^{e}	4.757^{c}	4.667^{b}	4.857^{d}	6.267^{f}	4.507^{a}	5.500^{e}	<.001	0.043	***

Key: DM – Dry matter; EE – ether extract; ADF – Acid detergent fiber; NDF - - Neutral detergent fiber; CP – crude protein, Ts=*Terminelie service* Means within a row followed by a different superscript are significantly different (P < 0.05). LSD= Least Significant Difference

A comparative analysis of bacterial morphology and arrangement in six plant samples was conducted (Table 2 and Figure 6). The results indicated a predominance of rod-shaped bacteria, particularly Gram-positive variants, in most samples. Cocci-shaped bacteria were less prevalent. Chain arrangements were observed in multiple samples, while single and cluster arrangements were less frequent. Spores were present in all samples, with the highest percentage found in Moringa. Flagella were observed in samples of Aloe, Vachellia, and *Terminalia sericea* leaves, suggesting the presence of motile bacteria. These findings highlight the diversity of bacterial populations associated with these plant species**.**

Upon physical analysis, no spoilage occurred upon opening the silage after eight weeks of ensiling. The color of the silage ranges from light green to olive green across different treatments, indicating potential differences in composition or processing methods (Table 3). Variations in smell are also observed among treatments, with some moderately sour and others slightly sour. However, the texture remains relatively consistent across treatments, with all exhibiting moderate moisture and a coarse texture.

Based on the data presented in (Table 3 and 4), the indigenous plants had a significant effect on the quality parameters of silages, as all p-values were less than 0.05. This indicates that the differences in means were statistically significant. The Least Significant values provided a threshold for determining significant differences between treatment means.

DISCUSSION

The demographic analysis of the participants provides valuable insights into the population's composition, particularly in terms of gender, age, education, and livestock experience, which are critical for understanding their interaction with indigenous plants and probiotic usage in livestock feed. The slight predominance of female participants (55%) over male participants (45%) highlights the important role that women play in livestock management and indigenous knowledge systems in Lupane District. This aligns with findings from previous studies (Mthi *et al*., 2018; Usman *et al*., 2022), which suggest that women in rural areas are often the primary caretakers of small livestock, such as goats and chickens, and possess valuable knowledge regarding local plant resources.

The age distribution, with 75% of participants aged between 36 and 55 years, suggests that the majority of respondents are experienced, active livestock keepers in their prime working years. This is supported by the fact that over 50% of participants reported more than 10 years of experience in livestock production. The combination of age and extensive experience likely contributes to a deeper understanding of traditional livestock management practices, including the use of indigenous plants for feeding.

The level of education varied among participants, with a notable 58% having completed only primary education and 25% having secondary education. The lower levels of formal education may influence the participants' ability to engage with and adopt scientific advancements in livestock nutrition, such as the use of probiotics. However, their strong foundation in practical, traditional knowledge compensates for this to some extent, especially regarding indigenous plant usage.

A significant proportion of participants (70%) demonstrated moderate to high knowledge of indigenous plants, suggesting that these plants are deeply embedded in the local culture and livelihood strategies. This knowledge could serve as a foundation for future interventions aimed at enhancing livestock nutrition through probiotic-enhanced feeds. On the other hand, the extremely low level of knowledge of probiotic products (only 1% of participants) reveals a major gap in awareness and utilization of modern feed-enhancement techniques. This gap highlights an opportunity for educational and extension services to introduce the concept of probiotics and their benefits in livestock feeding.

The results shows *that C. mopane* was the most frequently mentioned due to its dominance in Lupane district together with acacia species as well as *Terminalia sericea* as postulated by Mukungurutse *et al.* (2018) The ethnobotanical survey highlighted the significance of certain plant species in the local culture and traditional practices. The top five most frequently mentioned plants – *Colophosphermum mopane*, *Aloe Barbandesis* Miller, *Moringa oleifera*, *Vachellia karroo*, and *Terminalia sericea* - are all highly valued for their medicinal, nutritional, and cultural importance.

The laboratory experiment reveals compelling evidence that bacterial isolates from diverse plant sources; Moringa, Colophospermum mopane, Aloe, Vachellia, TS leaves, and TS seeds—exhibit a range of morphological and biochemical traits that strongly indicate probiotic potential. Notably, Gram-positive bacteria dominate these sources, comprising 60-80% of the isolates, and are renowned for their probiotic attributes, including antimicrobial activity and the ability to adhere to host cells (Sanders *et al.*, 2018). The prevalence of rod-shaped bacteria aligns perfectly with well-established probiotic strains like *Lactobacillus* and *Bifidobacterium* (Kumar *et al.*, 2019). Moreover, the chain arrangement of bacteria, observed in 60-100% of the samples, is a hallmark of probiotic organisms, facilitating their adhesion to host cells and biofilm formation (Li *et al.*, 2023). The presence of spores in 20-40% of the isolates further underscores their resilience, allowing them to endure harsh conditions and germinate when the environment is favorable (Elisashvili *et al.*, 2019). Additionally, flagella, found in 20-40% of the samples, enhances the bacteria's mobility towards optimal environments and improves their adherence to host cells (Chaban *et al.*, 2015). These findings strongly suggest that the bacterial isolates from these plant sources not only possess significant probiotic properties but also hold promise for further research into their potential health benefits.

The physical evaluation of silage treated with various plant materials revealed distinct differences in color, smell, and texture, reflecting the unique properties of each plant. Silage treated with Moringa and Vachellia exhibited a vibrant light green color, attributed to their high chlorophyll content (Kumar *et al.*, 2019). In contrast, Mopane and Aloe-treated silage displayed an olive-green hue, likely due to the presence of anthraquinones and other pigments (Moyo *et al.*, 2020). The TS leaves and seeds also produced a light green color, similar to that of Moringa and Vachellia, showcasing the consistency in visual appeal across these treatments.

All silage treatments emitted a moderately sour to slightly sour aroma, a natural indicator of the fermentation process (McDonald *et al.*, 2018). Notably, Moringa and Vachellia silage had a more pronounced sour smell, likely due to their elevated organic acid content (Girma *et al.*, 2019), enhancing their appeal as effective silage options.

In terms of texture, all treatments exhibited a moderately moist and coarse consistency, ideal for silage production (Ashbell *et al.*, 2019). Aloe-treated silage was slightly moister and coarser, likely due to the gel-like substance inherent in Aloe leaves (Raji *et al.*, 2020). Encouragingly, no spoilage was observed upon opening the silages, which may be attributed to the antimicrobial properties of certain plants, such as *Aloe vera* and *Terminalia sericea*, known to inhibit spoilage (Olajide *et al.*, 2019; Moyo *et al.*, 2020).

These physical characteristics are intricately linked to the biochemical composition of each plant material, influencing the fermentation process and ultimately determining the quality of the silage. This underscores the potential of utilizing specific plant materials to enhance silage production, ensuring both quality and preservation. The chemical analysis results indicate that silage treated with various plant materials may harbor probiotics, offering significant benefits for animal health and enhancing fermentation quality. Notably, the elevated dry matter content observed in mopane and Vachellia treatments suggests the potential presence of beneficial probiotics like *Lactobacillus* and *Bifidobacterium*, which are renowned for their ability to improve fermentation efficiency and increase dry matter retention (Sanders *et al*., 2018).

The higher ash content in the TS leaves treatment points to an increased mineral profile, which is essential for supporting probiotic growth (Raji *et al*., 2020). Vachellia's higher ether extract content indicates a richer fat composition, further promoting the proliferation of probiotics (Kumar *et al*., 2019). Furthermore, the lower ADF and NDF levels in the mopane treatment suggest reduced fiber content, facilitating easier colonization and fermentation by probiotics (Girma *et al*., 2019). The increased crude protein content in the Vachellia treatment also signifies a nutrient-rich environment conducive to probiotic development (Moyo *et al*., 2020). Moreover, the lower pH levels recorded in the *C. mopane* and Aloe treatments reflect higher acidity, which is favorable for probiotic growth (Ashbell *et al*., 2019).

In summary, these findings strongly suggest that incorporating different plant materials into silage can significantly influence the presence and growth of probiotics, ultimately enhancing fermentation quality and promoting better animal health. This highlights the importance of selecting appropriate plant materials to optimize silage production and its nutritional benefits.

CONCLUSION

This study offers compelling insights into the potential of indigenous plants to enhance the probiotic and fermentation properties of silage. Among the plants examined, *Colophospermum mopane* emerged as the most frequently cited in the survey, underscoring its cultural significance and dominance in the Lupane district. *Moringa oleifera* also stood out as a favored option, likely due to its well-documented nutritional benefits. Other plants such as *Aloe barbadensis* Miller, *Vachellia karroo*, and *Terminalia sericea* showed diverse yet meaningful applications within local traditions. Laboratory analyses of bacterial isolates from these plant materials revealed a predominance of Gram-positive, rod-shaped bacteria across all samples, with chain arrangements being the most common morphology observed. These characteristics, along with the presence of spores and flagella, strongly suggest that these bacteria may possess probiotic properties. The high prevalence of Gram-positive bacteria, particularly in Moringa and Mopane, aligns with the beneficial traits of well-known probiotic strains like *Lactobacillus* and *Bifidobacterium*, which are celebrated for their roles in enhancing digestion and overall gut health.

In the silage analysis, all treatments yielded silage with moderate to slightly sour aromas, consistent moisture levels, and a coarse texture, indicating successful fermentation without spoilage. The color variations among treatments likely reflect the distinct biochemical compositions of the various plants, while the absence of spoilage can be attributed to the antimicrobial properties of plants like Aloe and Terminalia.

Chemically, the silage treatments exhibited significant differences across key parameters, including dry matter (DM), ash, ether extract (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), and pH. Notably, Mopane and Vachellia demonstrated higher DM content, suggesting superior fermentation quality and potential probiotic activity. The presence of probiotics, such as *Lactobacillus*, likely contributed to the increased acidity observed in Mopane and Aloe, which may enhance silage preservation and promote better animal health. Overall, these findings highlight the promising role of indigenous plants in improving silage quality, offering a pathway to better animal nutrition and health through the strategic use of local resources.

RECOMMENDATIONS

Based on the results, *C. mopane* and Aloe plants showed promising results in terms of fermentation quality and nutrient preservation. Farmers are therefore recommended to consider incorporating these plants into silage production to improve feed quality and animal performance. Also, paying attention to factors such as chopping size, compaction, and ensiling duration can optimize the fermentation process and improve silage quality. It is recommended that further research be conducted to isolate and identify specific probiotic strains from indigenous plants like *Moringa* and *Colophospermum mopane*, and their benefits in livestock health should be explored through feeding trials. Optimizing silage formulations by varying proportions of these plants could improve fermentation quality and livestock productivity. Indigenous plants such as *Aloe*, *Vachellia*, and *Terminalia sericea*, with antimicrobial properties, should be investigated as natural alternatives to synthetic antimicrobials. Promoting the cultivation of these plants in semi-arid regions and documenting traditional knowledge related to their uses would be valuable for sustainable livestock management. Additionally, community-based silage production initiatives could empower smallholder farmers, and policy support should be considered to encourage the integration of indigenous feed resources into agricultural systems for enhanced food security and environmental sustainability. Also, molecular characterisation of the probiotic microorganisms which were isolated is recommended as this will build on knowledge on plant based probiotics.

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DECLARATION OF CONFLICT OF INTEREST.
The authors declare no conflicting interest in this paper.

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