Use of inoculant Sil-All and *Macrotyloma axillare* Legume on the Fermentation Characteristics and Quality of Maize Silage.

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Abstract

Tropical forage legumes are known as a potentially valuable source of protein for ruminant animals. Many forage species are underestimated due to the lack of knowledge of their nutritional feeding value. This study investigated the effects of the biological silage additive (Sil-all 4 × 4 inoculant) and *Macrotyloma axillare* legume inclusion on the fermentation quality, nutrient composition, and *in vitro* digestibility of whole crop maize silage. Sil-All provides rapid fermentation with maximum nutrient retention, it comprises four bacterial strains and four enzymes to enhance forage preservation during ensiling. Whole-crop maize (±665 g/kg DM) was ensiled for 45 days in a 5 kg air-tight polythene plastic bag. The bags were tied using twine to ensure airtightness and then stored at 22 – 25 °C. Three ensiling treatments were applied M: maize only, IM: inoculated maize and M+MA: maize + *Macrotyloma axillare* (80:20). The research was conducted at the North-West University research farm (Molelwane), Mafikeng area, North-West Province, South Africa. Both maize and *Macrotyloma axillare* legumes were planted as monoculture at North-West University research field. Replicate samples (n=5) were collected at pre-ensiling and post ensiling at day 45 for determination of fermentation and nutritive value. *In vitro,* DM degradability was determined using rumen cannulated Bonsmara cows. Inclusion of the legume *Macrotyloma axillare* in maize silage improved the nutritive value of the silage and, therefore, subsequently improved the fermentation substrate and lactic acid content. In all instances, the silages were deemed suitable for preservation based on their pH values ranging between 3.83 to 3.98. Crude protein was lower on IM, highest CP was observed on M+MA silage. Both Sil-All inoculant and *Macrotyloma axillare* legume were observed to reduce the fibre content of the silages. The inclusion of the legume improved the nutritive value and degradation of the silage compared to other treatments. An improvement in CP and OM content observed in M+MA silage suggested no necessity to supplement with concentrates and commercial minerals. Thus, *Macrotyloma axillare* (Java) legume qualified to compensate for the maize silage limiting nutrients without affecting its fermentation characteristics.

Keywords: Whole crop maize, *Macrotyloma axillare* legume, Sil-All inoculant, Silage

Introduction

Feed is the most critical input in livestock production and an adequate supply throughout the year is essential for any substantial and sustained expansion in livestock production (Michalk *et al*., 2019). However, due to erratic weather patterns, either low quality or the scarcity of animal feeds becomes a challenge. Given the change in climatic conditions, silage can be the greatest option for preserving fresh forage with minimal losses in quality. More research has been done on the significance of forage legumes as a valuable source of protein for ruminant animals (Washaya *et al*., 2018; Hawu *et al.*, 2022). *Macrotyloma axillare* is classified as a promising variety for use in further studies due to its higher seed yield, greater vegetative growth, nutritive value and greater tolerance to pests and diseases (Barbosa *et al*., 2019). In ruminant nutrition, maize silage is one of the most fed due to its nutritional higher energy. Maize represents sufficient traits of quality to produce excellent quality silage with estimated dry matter content (DM) that ranges between 20 - 35%. Furthermore, the whole maize plant has a high yield, high energy and is easy to ensile, it is best cultivated for silage making all over the world (Bernardi *et al*., 2019). However, it was reported that maize silage can be highly exposed and susceptible to nutrient losses and aerobic deterioration (Borreani *et al*., 2018) and low buffering capacity (Zang *et al*., 2022), and its most important challenge is the low protein content.

Legume plants, which can grow in the summer, might be a solution to dealing with this problem due to their high protein content and buffering capacity with low WSC, which may have a better effect on fermentation properties (Wang *et al*., 2017; Washaya *et al*., 2018). During dry periods, fermented forage species play an important role in ruminant nutrition offering improved feeding value, nitrogen fixation (N_2), minerals, vitamins, and protein (Chaudhary *et al*., 2022). The inclusion of *Macrotylona axillare* legume more specifically in maize silage as a source of protein may lead to enhanced growth performance in ruminants, particularly during winter as feed is scarce and of low quality. Previous studies described forage legumes as possible protein supplements for ruminants, as they comprise the high CP (150-300 g/kg DM), vitamins, as well as minerals required to develop ruminal microbes (Washaya *et al*., 2018).

The research done by Nkosi *et al*. (2019) highlighted that the utilization of inoculants has been used to increase the supply of WSC and enhance the quality of fermentation characteristics. The availability of many commercial inoculants has been used to boost the natural silage fermentation process, aerobic stability, and nutritional quality of silages. Numerous biological variables impact silage quality and nutritional value, including water-soluble carbohydrates (WSC) that are anaerobically transformed by lactic acid (LA) into organic acid, lowering the pH and inhibiting the spoiling activity (Nkosi *et al*., 2019).

During ensiling, lactic acid bacteria (LAB) break down the sugar compounds in the crop and lower the pH, eventually inhibiting further degradation by other bacteria (Nkosi *et al*, 2016). In this case, inoculants may be introduced to supply WSC and enhance the quality of fermentation properties. The use of inoculants, especially LAB, is to improve the efficiency of preserving the nutritional quality of the forage. However, the effect of LAB inoculants on forage fibre degradation is not consistent because LAB cannot effectively use fibre as an energy source to produce lactic acid (Nadeau *et al*., 2000) therefore, bacteria * enzymes inoculant may be of option to degrade maize silage cell wall and increase the availability of WSC. Sil-All inoculant is comprised of four bacterial strains and four enzymes, therefore, its use at ensiling will ensure the presence of sufficient bacteria and enzymes which may contribute to the elimination of undesirable fermentation through the release of fermentable sugars to produce more lactic acid and degrade fibre structure to improve the nutritive value of the silage.

Evidence shows that legumes improve the fermentation quality of the maize silage and may increase silage CP and mineral levels (Wang *et al*., 2017). Whereas, in most studies, biological inoculation inclusion has provided positive effects, whether on both the chemical and microbiological composition of the silage (Nkosi *et al*, 2016). Both can equally be used to improve the quality of the silage, however, on the other hand, the use of inoculants has been shown to be variable in effectiveness and, being expensive and difficult to apply, are not likely to be acceptable by small-holder farmers. Therefore, the ensiling of energy-rich cereal biomass with legume material could be a better option for producing protein-rich silage. Hence the current study was designed to determine the potential use *Macrotyloma axillare* legume to improve the fermentation pattern and quality of the whole crop maize silage over the inoculants. Studies of such mixed crop silages have been limited, but achievements have been shown when maize is ensilaged with legumes (Hawu e*t al*., 2022), therefore *Macrotyloma axillare* legumes as already been screened for their yield potential and forage quality (Barbosa *et al*., 2019) may be potential to improve maize silage properties.

Materials and methods

Study site, planting and harvesting

This experiment was conducted at the North-West University trial farm (Molelwane), Mafikeng (25.80°S and 25.50°E), North-West province of South Africa, at an altitude of about 1290 m above sea level. The prevailing temperature varies between 27 to 38°C throughout the summer and ranges between 11°C and 18°C throughout the winter season. The study area gets an average annual rainfall ranging from 200 to 450 mm yearly, with most of the rainfall occurring throughout the summer season. Maize plant was planted in February 2022 at North-West University Experimental Farm (Molelwane Farm), South Africa and harvested in late April 2022 with a Feraboli 945 forage harvester (Fondata Nel, Cremona, Italy) adjusted to achieve a 10 mm theoretical chop length. *Macrotyloma axillare* legume seeds were sourced from BRASUDA Seed Company, Pretoria, Gauteng Province, South Africa. *Macrotyloma axillare* seeds were planted as monoculture during the cropping season between August 2021 and July 2022. The legume was harvested at the flowering stage (13 weeks of age) of growth.

Ensiling procedure, experimental treatment and design

The Sil-All inoculant (2 g) was applied at a rate of 2 L/t of freshly chopped maize (2 g of Sil-All inoculant was dissolved in 2 L water 4 hours before application). To compensate for the water that was added to the treated silage, the other treatments were sprayed with 2 L of distilled water over a ton of fresh material to keep it at the same level of moisture as the treated silages. Maize plant (whole plant) and *Macrotylona axillare* legume were mixed at a ratio of 80:20. The description of silage mixtures was: M: maize only, IM: inoculated maize and M+MA: maize + *Macrotyloma axillare* legume. Three representatives of chopped maize and *Macrotyloma axillare* legumes were taken for the determination of pre-ensiling (before ensiling) chemical composition. Each of the three silages (5 kg) was independently placed in a closed air-tight polythene plastic bag. The squeezing and compaction of air in bags were done for the removal and elimination of air from plastic bags. The bags were then tied using twine to ensure airtightness and stored at 22 - 25°C. The silages were replicated 5 times and the opening of silages was done after 42 days of ensiling.

Chemical composition estimates and fermentation characteristics.

Chemical composition was determined for both pre-ensiling and post-ensiling samples. Dry matter (DM), organic matter (OM), and crude protein (CP) were determined according to methods of the Association of Official Analytical Chemists (AOAC, 2005). The samples were oven-dried for 12 hours at 105 °C to assess the DM content (method no. 930.15). ANKOM2000 fibre analyser (ANKOM Technology, New York) was used to determine NDF and ADF according to Van Soest *et al*. (1991), where samples (0.45 - 0.5 g) were refluxed with neutral and acid detergent solutions for 1 hour and 1 hour 15 minutes, respectively. A heat-stable bacterial α-amylase was included in the determination of NDF. Acid detergent lignin was evaluated by dissolving the cellulose in the ADF residue with 72% sulphuric acid. Ether extract (EE) was determined by an ANKOM Xt10 extraction system (ANKOM Technology, Macedon, and NY).

Cellulose and hemicellulose were calculated from the distinction between NDF, ADF and lignin.

Cellulose = ADF – ADL;
Hemicellulose = NDF - ADF

Non-fibre Carbohydrate (NFC) was calculated by adding CP, NDF, EE and ash in percentages and subtracting the sum from 100 (Malherbe, 2017). The formula for NFC is as follows:

NFC = 100 – (%CP + %NDF + %EE + %Ash)

Fermentation characteristics

A representative 40 g silage sample was taken from each silage to determine the fermentation characteristics. The 40 g silage sample (n = 5) was mixed with 360 mL of distilled water in a stomacher bag, homogenized for 4 min and the pH was determined immediately with a pH meter (Corning Model 4 pH-temperature, Ingold Messetechnik AG, Udorf, Switzerland). The sample was left at 10 °C for 24 h, homogenized for 4 min and filtered through a filter paper (Whatman No. 4). The filtrate was used to determine WSC and lactic acid (LA) concentrations. The WSC was determined by the phenol-sulphuric acid method and the LA was determined through colorimetric determination of lactic acid in biological material.

In vitro dry matter degradability (IVDMD) and ethical consideration

The ANKOM Daisy11 Incubator, which consists of a thermostatic chamber (39°C) with four rotating jars, was used to test the *in vitro* dry matter degradability of the silage samples according to ANKOM (2005). Silages (0.45 - 0.5 g) were weighed and packed in ANKOM F57 filter bags. The F57 filter bags were heat-sealed and put in the digestion jars. Buffer solutions were made and blended in a 1:5 ratio, with 1600 ml of the combined buffers poured into each jar and warmed at 39°C. A fistulated Bonsmara cow (weighing ± 550 kg) was used to collect rumen fluid. The cow was fed buffalo grass and *Macrotyloma axillare* legume diet. The collection of rumen fluid was done in two rewarmed thermos flasks and whisked together before being strained through two layers of warm muslin linen. Every jar received 40ml of rumen inoculum. Every jar received 400 ml of rumen inoculum, as well as ANKOM buffer (1600 mL) and F57 bags. The continuous purging of jars with carbon dioxide (CO_2) gas was done to maintain anaerobic conditions. The incubation was done at 39 °C. The F57 filter bags were withdrawn after 0, 2, 6, 12, 24, 36, and 48 hours of incubation. Following withdrawal, the bags were cleaned for 20 minutes with tap water using an ANKOM 2000 Fiber Analyzer, then oven dried at 60°C overnight before being weighed for DMD. This formula was used to calculate in vitro DM degradability:

$$\%IVDMD = [100 - (W3 - (W1 \times C1))] / (W2 \times DM) \times 100$$

where W1 was the tare weight of the bag, W2 was the weight of a sample, W3 was the final bag weight following in vitro treatment, and C1 was the blank bag correlation factor (final over dried weight / original blank weight).

The cow was cared for in accordance with guidelines established by the North-West University Animal Production Research Ethics Committee (approval number: NWU- 00817-21-A5) and the Federation of Animal Science Societies (FASS) for the care and use of farm animals in research and teaching.

Statistical analysis

Data on chemical composition, fermentation characteristics and *in vitro* dry matter degradability of silages was analyzed in a completely randomized design by ANOVA using the GLM procedure of SAS (2010). Data was fitted to the model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where: Yij was the individual observations of the ith treatment and the jth replicate, μ was the overall mean, Ti was the effect of the i^{th} treatment and Ɛij was the random variation or residual error.

Differences in treatment means were compared with the least significant difference (LSD) and significance was declared at $P<0.05$. The correlation coefficient was used to determine the degree of correlation between variables.

Results

Chemical composition of pre-ensiled maize, inoculated maize, and maize + Macrotyloma axillare

The results of the chemical composition of pre-ensiled maize only (M), IM, and M+MA mixes are presented in Table 1. There were significant differences ($P<0.05$) across all the treatments. Pre-ensiled M+MA had the highest ($P<0.05$) DM, OM, ADF and NFC contents when compared to other treatments ($P<0.05$). Pre-ensiled M and IM had similar ($P>0.05$) ash contents. The pre-ensiled M+MA had the highest ($P<0.05$) CP and NDF contents with the lowest values observed in inoculated maize. Pre-ensiled M had the highest ($P<0.05$) EE content when compared with other treatments with the lowest ($P<0.05$) observed on M+MA. Maize had the highest ($P<0.05$) ADL content (132.85 g/kg DM) with the lowest observed on pre-ensiled IM. Pre-ensiled M had the highest ($P<0.05$) cellulose content with the lowest ($P<0.05$) observed on M+MA. The M+MA had a lower ($P>0.05$) pH value.

Table 1. Chemical composition (g/kg DM, unless stated otherwise) of pre-ensiled maize, inoculated maize, and maize+*Macrotyloma axillare*

Parameter	Pre-ensiled treatments			SEM	PValue
	M	IM	M+MA		
DM (g/kg)	648.83[c]	657.38[b]	689.18[a]	2.23	0.001
Ash	86.46[a]	83.07[a]	61.62[b]	1.00	0.001
OM	562.36[c]	574.31[b]	627.56[a]	2.79	0.001
CP	94.50[b]	80.20[c]	116.20[a]	0.51	0.001
EE	43.08[a]	38.81[b]	11.05[c]	0.96	0.001
NDF	502.95[a]	501.80[a]	463.50[b]	19.91	0.001
ADF	263.50[b]	249.50[b]	285.50[a]	33.92	0.091
ADL	132.85[a]	132.01[a]	105.18[b]	0.64	0.001
NFC	31.37[c]	34.27[b]	41.68[a]	0.17	0.001
Cel	120.70[a]	52.84[c]	104.84[b]	1.42	0.001
Hemi	215.00[a]	229.00[a]	118.00[b]	12.06	0.013
pH	5.73[a]	5.45[a]	4.60[b]	0.089	0.006

[a,b,c] Means in the same row with different superscripts are different (P<0.05), DM: dry matter, OM: organic matter, EE: ether extract, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, NFC: non-fibre carbohydrates, Cel: cellulose, Hemi: hemicellulose, M: Maize only; IM: inoculated maize; M+MA: Maize+ *Macrotyloma axillare*; SE: standard error; n=5.

Chemical composition of ensiled maize, inoculated maize, and maize + Macrotyloma axillare

The results of the chemical composition of silages are presented in Table 2 below. There were significant differences (P<0.05) between the treatments on measured parameters. The IM silage had the highest (P<0.05) DM and ash content (687.7 and 124.9 g/kg DM) as compared with other silages. The M+MA silage had a higher (P<0.05) CP content than other silages. The M and IM silages had the highest (P<0.05) ADF and NDF contents, respectively. The M silage had the highest (P<0.05) ADL content (168 g/kg DM) when compared with other silages. Furthermore, M+MA silage had the highest (P<0.05) LA and WSC contents (7.26 and 3.11 g/kg DM) when compared with M and IM silages, respectively. The IM silage had the highest (P<0.05) pH than the other silages.

In vitro dry matter degradability of silages within each incubation time

The results on *in vitro* dry matter digestibility (IVDMD) varied significantly (P<0.05) among the silage treatments (Table 3). Inoculated maize (IM) and M+MA silages recorded the higher (P <0.05) IVDMD at 2, 24, 36, and 48 hours with maize silage showing the lowest (P<0.05) values.

Discussion

Dry matter is the basic indicator for desirable silage, the recommended DM content should not be less than 300 g/kg for considered beneficial preservation/silage making (Fang *et al.*, 2022). Any value less than 300 g/kg will inhibit clostridial bacterial activity which will lead to more acetic acid

Table 2. Chemical composition (g/kg DM, unless otherwise stated) and fermentation characteristics of experimental silages after 42 days of ensiling

Parameters	Ensiled treatments			SE	P-Value
	M	IM	M+MA		
DM (g/kg)	615.2^{b}	687.7^{a}	641.5^{b}	8.88	0.001
Ash	133.9^{a}	124.9^{b}	115.9^{c}	1.56	0.001
OM	481.3^{c}	502.7^{b}	525.6^{a}	9.57	0.001
CP	84.9^{b}	79.7^{c}	102.7^{a}	0.30	0.001
EE	28.4^{a}	19.8^{b}	31.0^{a}	1.01	0.001
NDF	684.0^{a}	682.3^{a}	612.5^{b}	1.57	0.001
ADF	459.7^{a}	465.9^{a}	412.9^{b}	1.68	0.001
ADL	168.4^{a}	122.4^{c}	135.6^{b}	1.62	0.001
NFC	262.3^{c}	424.7^{a}	316.9^{b}	0.27	0.001
Cel	290.9^{b}	343.5^{a}	277.2^{c}	2.28	0.001
Hemi	224.7^{a}	216.4^{b}	199.7^{c}	1.51	0.001
Fermentation characteristics					
LA, g/kg	3.14^{c}	4.01^{b}	7.26^{a}	0.09	0.001
WSC g/kg	2.02^{b}	1.52^{c}	3.11^{a}	0.68	0.001
pH	3.83^{b}	3.98^{a}	3.85^{b}	0.21	0.004

[a,b,c] Means in the same row with different superscripts are different ($P<0.05$), DM: dry matter, OM: organic matter, EE: ether extract, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, NFC: non-fibre carbohydrates, Cel: cellulose, Hemi: hemicellulose; M: Maize; IM: inoculated maize; M+MA: maize + *Macrotyloma axillare*; LA: Lactic Acid; WSC: water-soluble carbohydrates; SE: standard error. n=5.

Table 3. *In vitro* dry matter digestibility of maize, inoculated maize and maize + *Macrotyloma axillare* silages after 0, 2, 6, 12, 24, 36, and 48 hours of incubation (g /kg DM)

Trt	M	IM	M+MA	SE	P-Value
IVDMD02	205.6^{b}	247.7^{a}	238.2^{a}	7.2	0.001
IVDMD06	230.4^{b}	262.9^{b}	327.8^{a}	7.3	0.001
IVDMD12	479.3^{a}	303.0^{b}	481.4^{a}	6.8	0.001
IVDMD24	366.2^{b}	579.1^{a}	578.2^{a}	6.5	0.001
IVDMD36	529.6^{b}	636.4^{a}	646.1^{a}	7.9	0.001
IVDMD48	600.0^{b}	712.6^{a}	720.3^{a}	8.6	0.001

[a,b] Means in the same column with different superscripts are different ($P<0.05$), M: Maize; IM: inoculated maize; M+MA: Maize+ *Macrotyloma axillare;* Trt: treatment, IVDMD: *In vitro* dry matter digestibility SE: standard error

production during fermentation, which will affect the quality of the silage, resulting in DM losses and silage of low nutritional value (Fang *et al*., 2022). The DM content of maize only, inoculated maize and maize + *Macrotyloma axillare* were greater than 600 g/kg DM but less than 700 g/kg DM. As noted by Kanengoni *et al*. (2016), such a high DM potentially impairs effective ensiling by limiting the growth of lactic acid bacteria and the extent of fermentation. However, Colombari *et al.* (2001) reported no differences in alfalfa silage quality ensiled at either low or high DM (360 and 580 g/kgDM). The CP content is the compositional factor that affects the nutritional quality of silages (Hawu *et al*., 2022). Related to this study, Addah *et al*. (2011) also compared the fermentation characteristics, aerobic stability and nutritive value of barley and corn silages ensiled with or without a mixed bacterial inoculant. In this study, treatment with Sil-All inoculant reduced the CP content of maize. This was not consistent with other studies in which microbial inoculation improved the CP content of silage (Nkosi *et al*., 2019). Furthermore, the presence of enzymes throughout ensiling should lead to degradation of protein, vitamins and enzymes (Yang *et al*., 2021). The reduction of CP with inoculant Sil-All treatment is difficult to explain as microbial/ biological inoculation has been reported to reduce proteolysis, therefore, improving the CP content of silage (Nkosi *et al.*, 2019). Crude protein was observed to increase with the inclusion of *Macrotyloma axillare*. This was in line with most studies done on maize–legume silage, which highlighted the inclusion of legumes in maize silage making to be a feasible strategy to increase the CP of silage (Wang *et al*., 2017; Hawu, *et al*., 2022). The CP content (84.9 g/kg DM) current study on maize only silage was greater than the threshold (70 g/kg DM) which meets the requirement to promote the activity of optimum ruminal activity (van Soest, 1994). The NDF consists of total fibre contents which contain cellulose, hemicellulose, and lignin. The use of Sil-All inoculation did not affect the NDF and ADF of maize silage of the present study, both NDF and ADL values similar to maize only silage and higher than those of maize + *Macrotyloma axillare* silage. The non-significant results observed with the use of Sil-All inoculant in the current study were in line with other studies that observed no significant differences in cell wall content of the silage with the use of LAB+enzyme mixture inoculants (Moselhy *et al*., 2015). However, contrary to the current study Nkosi *et al*. (2019) reported decreased cell wall contents of silages with the use of LAB+enzyme mixture inoculants. The non-significant differences with the inclusion of bacteria and enzyme inoculant are difficult to explain as studies suggest enzymes to - improve silage fermentation and alter the fibre content of silages (Nkosi *et al*., 2016; Nadeau *et al*., 2000). However, this could be due to the length of ensiling time and moisture level of forage. In the current study, the pre-ensiling forage was considerably drier (over 500g/kg DM) than those used in many other studies (usually less than 300g/kg DM), and moisture activity may not have been adequate for optimal enzyme activity.

Lower ($P<0.05$) NDF was observed on maize + *Macrotyloma axillare* legume. In the current research, the ADF of silages ranged between 412.9 to 465.6 g/kg DM. Lower concentrations of NDF and ADF are associated with improved feed intake and digestibility, these current findings represent an improvement of silage fibre content through the inclusion of either *Macrotyloma axillare* or Sil-All inoculant in maize silage**.** Possibly the NDF and ADF content in maize + *Macrotyloma axillare* silage was lower because the inclusion of either the inoculant or legume weakened the fibre structure of maize. The acid detergent fibre content of maize in the study of Hawu *et al*. (2022) was 385.7 g/kg DM which was relatively lower than in the current study. The variation can be accounted for by many factors such as harvesting time, maize variety and DM content at ensiling.

In the primary and secondary walls, with the advancing maturity of forages, cells insert non-carbohydrate material which is known as lignin. Lignin is known as the primary skeleton of the plant cell. Lignin is the major chemical constituent contributing to lowered digestibility of the feedstuffs, by inhibiting cellulose and hemicellulose. In this research, ADL was high in maize silage, however, the values observed on maize silage in the current study were more comparable to those reported by Hawu *et al* (2022) who reported maize silage ADL of 140 g/kg DM which was however still lower (168.4 g/kg DM) than of these current findings. This can be accounted for by the differences in harvesting time and maize variety. Furthermore, the hemicellulose (Hemi) content of maize only silage was higher than that of inoculated and legume maize silage. The results obtained in the current study were similar to the results obtained by Wang *et al*. (2017). The author reported hemicellulose of maize silage was higher than of legume maize silage. The cellulose (Cel) content of inoculated maize silage (343.5 g/kg DM) in the current study was higher than that of maize and maize-*Macrotyloma axillare* silages. Maize: *Macrotyloma axillare* silage had the lowest Cel and Hemi content which is attributed to the general concept that legumes have lower cellulose and hemicellulose content. This had a further impact on the NFC. The NFC are an important parameter to measure as the indication of the energy value of forages and energy; it is the major daily nutrient required for maintenance and production. The NFC for maize only silage was lower than that of inoculated maize and maize-*Macrotyloma axillare* silage. Souza *et al*. (2014) reported NFC of maize silage of 373.3 g/kg DM which was higher than the current study (262.3 g/kg DM). The increase in NFC with the inclusion Sil-All inoculant was expected as the treatment of maize with bacteria enzyme inoculant degrades the structural carbohydrates of corn straw into NFCs (Guo *et al*., 2022). This agreed with Souza *et al*. (2014) who observed a relatively low NFC (316.9 g/kg DM) in maize alone silage. The NFC observed in maize alone silage and inoculated maize silage were outside the recommended ranges, with maize silage having the lowest and inoculated maize silage having the higher values. Recommended dietary allowances for NFC are between 35%-40% (Schwab *et al*. 2006). Feeding silage with a higher NFC content usually will require the use of high-fibre byproducts in the concentrate so that the upper limits on dietary NFC are met, while lower NFC content silage will require high-concentrate products to meet the upper limits of dietary NFC. The OM content of maize-*Macrotyloma axillare* silages in the current study was higher than those observed on maize only silage, while the ash content of silages in the current study ranged between 135.8 and 160.9 g/kg DM. The ash values in this study were found to be higher than the range (59.0-70.0 g/kg DM) reported by Edson *et al*. (2018) on maize and maize-legume silages. This could be an added benefit when supplemented to ruminants fed on roughages of poor quality that are lacking minerals. The ash content found in feeds plays a vital role in stimulating balanced animal growth (Hawu *et al*., 2022), this suggests that there may be no necessity to supplement with commercial minerals if these silages are used. There is a need to analyse for minerals required by ruminants to determine the adequacy of these silages.

Organic matter is defined as the loss of weight of dry matter when combusted. According to Ginwal *et al*. (2019), ether extract is a combination of fat-soluble substances found in the feed sample. Ether extract for both maize silage (28.4 g/kg DM) and maize-*Macrotyloma axillare* silage (31.0 g/kg DM) was similar and that of inoculated maize silage (19.8 g/kg DM) was lower in the current study. Ether extract content of maize silage reported by Hawu *et al*. (2022) was 24.9 g/kg DM which was more comparable to the one observed in the current study. Low EE content found in inoculated maize silage of the current study is preferable, as high EE content suppresses the feed intake of animals (Mciteka, 2008).

The pH is one of the main indicators for good and stable silage (Nkosi *et al*., 2016). The pH range of 3.7 to 4.2 is considered beneficial for whole maize preservation (Kanengoni *et al*., 2019). In the current study the pH of maize alone, inoculated maize and legume maize silages were 3.83, 3.98 and 3.85, respectively and this clearly showed good silage preservation. Water-soluble carbohydrates are regarded as essential substrates for the growth of LAB for proper fermentation (Nkosi *et al*., 2016), and low levels may restrict LAB growth. Oliveira *et al*. (2017) indicated that inoculation decreases the WSC concentration of silages. Addah *et al*. (2011) reported WSC of maize (M) and inoculated maize (IM) silages at 42 days after ensiling in the range of 3 - 4 g/kg DM while in the current study, they were between 1-3 g/kg DM, the differences may be due to variations in chop length, harvesting (Oliveira *et al*., 2017). After 42 days of ensiling, water-soluble carbohydrates were *Macrotyloma axillare* legume maize silage but lower than the recommended values, this improved the lactic acid fermentation bacteria in silage, with an improved silage fermentation. Legume maize silage had a higher LA content when compared to maize and inoculated maize silages in the current study. However, the values were lower than those reported by Hawu *et al*. (2022). Baghdadi *et al*. (2016) reported similar LA values of maize and legume maize silages to the current study. The significant increase observed in LA of legume maize silage is consistent with the studies done by Baghdadi *et al*. (2016) and Hawu *et al*. (2022). The WSC is considered the major component for the vital growth of lactic acid to enhance fermentation. The improvement and growth of lactic acid content is the major reliable indicator for microbial addictive achievement in improving silage quality. Both treatments of Sil-All inoculant and legume in whole maize crops improved LA concentration. Achieving an increase in the content of LA is the most reliable indicator for the success of Sil-All inoculant and *Macrotyloma axillare* legume in improving silage quality resulting in a drop of silage pH, with less losses of dry matter and nutritive value of forage during storage. Nonetheless, the LA value observed for maize only and inoculated maize silage was lower than that of M+MA silage in the current study, and both maize and inoculated maize silages are in line with the study completed by Keleş & Yazgan (2011).

The IVDMD of silages was determined at 2, 6, 12, 24, 36, 48, and 72 hours of incubation. In the current study, the degradation of substrates of all the forages used increased as the incubation period increased, and this was also observed by Hawu *et al*. (2022). A higher IVDMD indicates that an animal reacts positively to the silage, it is of a good/excellent quality and shows improved production. The study revealed maize + *Macrotyloma axillare* silage had the highest IVDMD across all withdrawal periods, with maize silage having the lowest. This can be explained by the higher ADL content observed in maize silage compared to other treatments, as the lignin greatly reduces the degradability and IVDMD. The inclusion of *Macrotyloma axillare* legume during fermentation improved the CP in silage, CP is highly related to the degradability of forage through improved microbial population. Hawu *et al*. (2022), also highlighted the increased in *in vitro* degradability of legume maize silage. Furthermore, the inclusion of *Macrotyloma axillare* legume in the diet reduced NDF and ADL content of the silage, which factors into improving the IVDMD. A higher IVDMD of maize-*Macrotyloma axillare* silage at the mentioned hours indicates that legume improves degradability and can be used to improve the nutrient content of feedstuffs with low nutrient content especially those with low crude protein. In the current study, the inclusion of Sil-All inoculant and *Macrotyloma axillare* legume in maize resulted in improved degradability of silages. This could be linked with the higher CP content since it is highly digestible.

Conclusion

Equally Sil-All inoculant and *Macrotyloma axillare* legume has proved to enhance the IVDMD of the silages. In all instances the silages were deemed suitable for preservation based on their pH values however, in general, inclusion of the *Macrotyloma axillare* legume to whole maize crop did not negatively affect the quality and fermentation characteristics of the silage. The inclusion of *Macrotyloma axillare* legume leaves stimulated WSC production which is the substrate used by lactic acid to influence the bacterial community for good quality silage with high buffering capacity.

Recommendations

Further researchers are required to investigate the combined effect of the inclusion of *Macrotyloma axillare* legume and Sil-All inoculants on maize silage characteristics and nutritive value. More studies are necessary to evaluate the effect of inoculant Sil-All and *Macrotyloma axillare* legume inclusions in maize silages on the performance of small ruminants.

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References

Addah, W., Baah, J., Groenewegen, P., Okine, E.K. & McAllister, T.A. 2011. Comparison of the fermentation characteristics, aerobic stability and nutritive value of barley and corn silages ensiled with or without a mixed bacterial inoculant. *Can. J. Anim. Sci*. 91(1): 133-146.

AOAC. 2005. Method number 984.13. *In Official Methods of Analysis of AOAC International*. 16th edition. Association of Official Analytical Chemists, Arlington, VA, USA.

Baghdadi, A., Halim, R.A., Radziah, O., Martini, M.Y. & Ebrahimi, M. 2016. Fermentation characteristics and nutritive value of corn silage intercropped with soybean under different crop combination ratios. *J. Anim. Plant Sci.* 26(6): 1710-1717.

Barbosa, H.Z., Batista, K., de Andrade Gimenes, F.M., Gerdes, L., Giacomini, A.A., de Mattos, W.T. *et al*. 2019. Yield responses of *Macrotyloma axillare* (family Fabaceae) to combinations of doses of phosphorus and calcium. *Seminar: Agric. Sci.* 40(6): 2561-2570.

Bernardi, A., Härter, C.J., Silva, A.W., Reis, R.A. & Rabelo, C.H. 2019. A meta-analysis examining lactic acid bacteria inoculants for maize silage: Effects on fermentation, aerobic stability, nutritive value and livestock production. *Grass Forage Sci*. 74(4): 596-612.

Borreani, G.I.O.R.G.I.O., Tabacco, E.R.N.E.S.T.O., Schmidt, R.J., Holmes, B.J. *et al*. 2018. Silage review: Factors affecting dry matter and quality losses in silages. *J. Dairy Sci*. 101(5): 3952-3979.

Chaudhary, S., Dhanker, R., Kumar, R. & Goyal, S. 2022. Importance of legumes and role of Sulphur oxidizing bacteria for their production: a review. *Legume Res*. 45(3): 275-284.

Colombari, G.; Borreani, G. & Crovettte, G.M. 2001. Effect of Ensiling Alfalfa at Low and High Dry Matter on Production of Milk Used to Make Grana Cheese. *J. Dairy Sci*. 84 (11): 2494–2502

Edson, C., Takarwirwa, N.N., Kuziwa, N.L., Stella, N. & Maasdorp, B. 2018. Effect of mixed maize-legume silages on milk quality and quantity from lactating smallholder dairy cows. *Trop. Anim. Health Prod*. 50: 1255-1260.

Fang, D., Dong, Z., Wang, D., Li, B., Shi, P., Yan, J., *et al.*. 2022. Evaluating the fermentation quality and bacterial community of high-moisture whole-plant quinoa silage ensiled with different additives. *J. Appl. Microbiol*. 132(5): 3578-3589.

Ginwal, D.S., Kumar, R.A.K.E.S.H., Ram, H.A.R.D.E.V., Meena, R.K. & Kumar, U. 2019. Quality characteristics and nutrient yields of maize and legume forages under changing intercropping row ratios. *Indian J. Anim. Sci*. 89 (3): 281-286.

Gülümser, E., Mut, H., Başaran, U. & Doğrusöz, M.Ç. 2021. An assessment of ensiling potential in maize x legume (soybean and cowpea) binary mixtures for yield and feeding quality. *Turkish J. Vet. Anim. Sci*. 45(3): 547-555.

Guo, W., Guo, X.J., Xu, L.N., Shao, L.W., Zhu, B.C., Liu, H., *et al.* 2022. Effect of whole-plant corn silage treated with lignocellulose-degrading bacteria on growth performance, rumen fermentation, and rumen microflora in sheep. *Animal*. 16(7): 100576.

Hawu, O., Ravhuhali, K.E., Mokoboki, H.K., Lebopa, C.K. & Sipango, N. 2022. sustainable use of legume residues: effect on nutritive value and ensiling characteristics of maize straw silage. *Sustain*. 14 (11): 6743.

Kanengoni, A.T., Nkosi, B.D., Chimonyo, M., Ndimba, B. & Dzama, K. 2016. Effects of whey, molasses and exogenous enzymes on the ensiling characteristics, nutrient composition and aerobic stability of maize cobs. *S. Afr. J. Anim. Sci*. 46(2):113-120.

Keleş, G. & Yazgan, O. 2011. Fermentation characteristics of maize silages ensiled with lactic acid bacteria and the effect of inoculated baled maize silages on lamb performance. *J. Kafkas Univ. Vet. Fak. Derg*. 17(2): 229-234.

Michalk, D.L., Kemp, D.R., Badgery, W.B., Wu, J., Zhang, Y. & Thomassin, P.J. 2019. Sustainability and future food security—A global perspective for livestock production. *Land Degrad. Dev*. 30(5): 561-573.

Moselhy, M.A., Borba, J.P. & Borba, A.E. 2015. Improving the nutritive value, *in vitro* digestibility and aerobic stability of *Hedychium gardnerianum* silage through application of additives at ensiling time. *Anim. Feed Sci. Technol*. 206: 8-18.

Nadeau, E.M.G., Russell, J.R. & Buxton, D.R. 2000. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. *Anim. Sci. J*. 78(11): 2980-2989.

Nkosi, B.D., Meeske, R., Langa, T., Motiang, M.D., Modiba, S., Mkhize, N.R. *et al.*

2016. Effects of ensiling forage soybean (*Glycine max* (L.) Merr.) with or without bacterial inoculants on the fermentation characteristics, aerobic stability and nutrient digestion of the silage by Damara rams. *Small Rumin. Res*. 134: 90-96.

Nkosi, B.D., Meeske, R., Muya, M.C., Langa, T., Thoma, R.S., Malebana, I.M.M., *et al*. 2019. Microbial additives affect silage quality and ruminal dry matter degradability of avocado (Persia Americana) pulp silage. *S. Afr. J. Anim. Sci*. 49 (6): 998 – 1007. https://doi.org/10.4314/sajas.v49i6.3

Oliveira, A.S., Weinberg, Z.G., Ogunade, I.M., Cervantes, A.A., Arriola, K.G., Jiang, Y., *et al*. 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. *J. Dairy Sci*. 100 (6): 4587-4603.

SAS, 2010. Users Guide: Statistics. Version 9.3. SAS Institute, Cary, NC, USA.

Schwab, E, C., Schwab, C, G., Shaver, R.D., Girard, C.L., Putnam, D.E., & Whitehouse, N.L. 2006. Dietary forage and nonfiber carbohydrate contents influence B-vitamin intake, duodenal flow, and apparent ruminal synthesis in lactating dairy cows. *J. Dairy Sci*. 89(1): 174-87. DOI: 10.3168/jds.S0022-0302(06)72082-3

Souza, W.F., Pereira, O.G., Ribeiro, K.G., Santos, S.A. & Valadares Filho, S.C. 2014. Intake, digestibility, nitrogen efficiency, and animal performance of growing and finishing beef cattle fed warm-season legume (*Stylosanthes capitata* plus *Stylosanthes macrocephala*) silage replacing corn silage. *J. Anim. Sci*. 92(9): 4099-4107.

Van Soest, P.V., Robertson, J.B. & Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. 74(10): 3583-3597.

Wang, S., Yuan, X., Dong, Z., Li, J., & Shao, T. 2017. Effect of ensiling corn Stover with legume herbages in different proportions on fermentation characteristics, nutritive quality and in vitro digestibility on the Tibetan plateau. *Grassl. Sci.* 63: 236–244. doi: 10.1111/grs.12173

Washaya, S., Mupangwa, J. & Muchenje, V. 2018. Chemical composition of Lablab purpureus and Vigna unguiculata and their subsequent effects on methane production in Xhosa lop-eared goats. *S. Afr. J. Anim. Sci*. 48 (3): 445-458.

Xing, L., Chen, L.J. & Han, L.J. 2009. The effect of an inoculant and enzymes on fermentation and nutritive value of sorghum straw silages. *Bioresour. Technol*. 100(1): 488-491.

Yang, H., Wang, B., Zhang, Q., Cheng, H. & Yu, Z. 2021. Improvement of fermentation quality in the fermented total mixed ration with oat silage. *Microorganisms*. 9(2): 420.