



**Legumes in maize straws: nutritive value, palatability indices and
silage fermentation parameters**

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Declaration

I, Onke Hawu, declare that this work was not submitted to any university other than North-West University and consists of my original work under the supervision of Prof. H.K Mokoboki, Dr K.E Ravhuhali, and Dr C.K Lebopa. All assistance has been duly credited to the creation of this document and all the references mentioned herein.

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Journal articles from the dissertation

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Abstract

Legume leaves from lablab and cowpea can be incorporated in ruminants' diets due to their high feeding value. They are rich in crude protein (CP), vitamins and minerals when compared to grasses that usually deteriorate during dry periods. The objective of this study was to determine the effect of supplementing legume leaves in maize straws on nutritive value, palatability indices and silage fermentation parameters. The study was conducted at the North-West University farm (Campus) and research farm (Molelwane), Mafikeng, North West province of South Africa. The study sites are located in the semi-arid region with geographical coordinates (25°49'22" S and 25°36'54" E) and (25°47'27" S and 25°37'18" E). Four legume species including *Lablab purpureus* and 3 *Vigna unguiculata* varieties (Dr Saunders, Betswit, and indigenous cowpea) were planted in monoculture in the experimental field, at 4 replications for every species. Maize (*Zea mays*) was planted in a monoculture. For the palatability experiment, each of five male boer goats was provided with four feeding troughs, with four different feeds (maize straws and 3 legumes (lablab and 2 cowpeas - Dr Saunders, and Betswit). Indigenous cowpea was excluded for palatability trial due to little quantity. For silage, the proportion of legume to maize was 20:80. Treatments were as follows, maize-lablab (ML) (20:80%), maize-indigenous cowpea (MI) (20:80%), maize-betswit (MB) (20:80%) and maize-Dr Saunders (MD) (20:80%), each with molasses (2%) to enhance fermentation. The controls were maize, without (negative control) or similarly treated with molasses (positive control). Molasses was diluted with distilled water at 1:2. The silages were opened after 45 days. The leaves and maize straw were analyzed for chemical composition and *in vitro* dry matter degradability (IVDMD) while silages were analyzed for chemical composition, fermentation characteristics, aerobic stability and IVDMD. All data were subjected to a one-way analysis of variance (SAS, 2010). The highest ($P<0.05$) CP (230.7 g/kg DM) content was recorded for Dr Saunders cowpea. The lowest ($P<0.05$) acid detergent lignin (ADL) content (116.7 g/kg DM)

was obtained from indigenous cowpea. Lablab had higher ($P<0.05$) total phenols (0.36 g/kg DM) than all forages. Lablab and indigenous cowpea had the highest ($P<0.05$) metabolizable energy (ME) content. Highest ($P<0.05$) relative feed value (RFV) was recorded for lablab. The highest ($P<0.05$) *in vitro* dry matter degradability (IVDMD) was recorded for Dr Saunders and lablab. Lablab had a higher palatability index ($P<0.05$) when compared to other diets. Intake ($r^2=0.955$) and palatability indices ($r^2=0.971$) were positively and significantly ($P<0.05$) predicted from total phenolics. For the chemical composition of the silage, maize-molasses had the highest DM and hemicellulose content than all other silages. Maize–Dr Saunders cowpea had the highest ($P<0.05$) ash, and ether extract (EE) content when compared to all other silages. Maize-lablab silage had the highest ($P<0.05$) CP content (116.0 g/kg DM). Maize had the highest ($P<0.05$) neutral detergent fibre (NDF) content (619.2 g/kg DM). The ML and MD had the highest ($P<0.05$) ADF content (394.7 and 393.6 g/kg DM respectively) than other silages. Maize had the highest content of ADL content (140.0 g/kg DM). The ML had the highest ($P<0.05$) cellulose (270.3 g/kg DM). The highest ($P<0.05$) IVDMD was recorded for ML silage from 24hr up to 72hrs. The highest ($P<0.05$) pH was recorded for MB. All silages had similar ($P>0.05$) concentration of LA. The MM had the highest ($P<0.05$) content of WSC and ML had the least ($P<0.05$) content of WSC. The ML had the highest ($P<0.05$) LAB counts than all other silages. The yeast and mould counts of all silages were similar ($P>0.05$). A higher ($P<0.05$) carbon dioxide (CO₂) was recorded for MB than all silages. Except for total phenolics, all chemical composition parameters poorly predicted intake and palatability index. Although lablab had better values in most parameters measured, all legumes can be highly recommended to supplement low-quality roughages such as maize straws and grasses.

Keywords: goats, intake, legumes, nutritive value, palatability, regression, silage

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Dedication

This work is dedicated to my little brother Banele Hawu, my late parents, and my late sister.

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List of abbreviations

ADF:	Acid Detergent Fibre
AOAC:	Association of Official Analytical Chemists
CO ₂ :	Carbon Dioxide
CP:	Crude Protein
EE:	Ether Extract
IVDMD:	<i>In Vitro</i> Dry Matter Degradability
LA:	Lactic Acid
M:	Maize
MB:	Maize - Betswit
MD:	Maize – Dr Saunders
MI:	Maize - Indigenous Cowpea
ML:	Maize - Lablab
MM:	Maize - Molasses
N:	Nitrogen
NDF:	Neutral Detergent Fibre
OM:	Organic Matter
SAS:	Statistical Analyses System
WSC:	Water Soluble Carbohydrates

Chapter 1: General Introduction

1.1 Background

Rangelands play a crucial role in South Africa's ruminant production, as this is the primary feed resource that is cheaply available. In southern Africa, ruminant production, however, is restricted by the low value and amount of dry seasonal forage available (Ngongoni *et al.*, 2006; Shinde & Mahanta, 2020), which are exacerbated by the poor management (Singh *et al.*, 2005; Shinde & Mahanta, 2020; Msiza *et al.*, 2021) and by climate change (Meissner *et al.*, 2013; Washaya *et al.*, 2018; Motsei *et al.*, 2020). Available forage in the rangelands during the dry season is fibrous and contains low crude protein (CP) (Brown *et al.*, 2016; Mnisi & Mlambo, 2017). The CP levels are insufficient for rumen microbial protein synthesis, leading to weight loss during the winter period.

Small ruminants such as sheep and goats play a very significant financial, societal and traditional roles for rural households as they help boost the family's income and well-being. Small ruminants' aids in the food supply, household nourishment, income, asset investments, soil quality, livelihoods, ranch diversification and maintainable agricultural production, employment, ceremonial purposes as well as social prestige (Brown *et al.*, 2018; Shinde & Mahanta, 2020).

Preservation of forage crops should be an important part of ruminant production given the growth of small-scale intensive systems and the increasing concentrate feedstuffs prices (Mapiye *et al.*, 2007). In South Africa, numerous legumes have been produced for forage production, which can be grown and stored as hay or silage for feeding throughout the dry season in the marginal rainfall areas (Mapiye *et al.*, 2007; Gwanzura *et al.*, 2012). Silage has historically been produced primarily from maize (*Zea mays*) with less from sorghum (*Sorghum bicolor*) and pasture grasses (Titterton *et al.*, 2002; Gwiriri *et al.*, 2016). Maize is the favoured cereal crop for silage, generating great

yields (14.7 t DM/ha) and high energy (10.2 MJ/kg DM) in the high rain subtropical regions of South Africa (Titterton, 1997). The main shortcoming, however, is its low content of CP, varying from 69-88 g/kg DM (Contreras-Govea *et al.*, 2009; Ivan *et al.*, 2018; Uher *et al.*, 2019). In pure maize silage, the CP content improved from 73 g/kg DM to a maximum of 105 to 136 g/kg DM when combined with soybean at 25 and 50% respectively (Stella *et al.*, 2016).

The CP and fibre content influence the intake and palatability of a feed (Deng *et al.*, 2017; Idan *et al.*, 2020). Mpanza (2015) and Osuga *et al.* (2020) mentioned that, when forages mature, there is a reduction in CP, increment in fibre content, which adversely influence palatability, intake and rumen degradability. Both palatability and rumen degradability have been reported to be affected by the presence of tannins (Vahdani *et al.*, 2014). The low degradability of plant species high in fibre as well as tannins is caused by the decreased penetrative capacity to rumen microflora through lignified plant cells as well as tannins' precipitation influence on microflora and enzymes (Mahwasane, 2018). Therefore, the main objective of the present study was to investigate the effect of legume leaves in maize straws on nutritive value, palatability indices and silage fermentation parameters.

1.2 Problem statement

Sheep and goats can boost the socio-economic status of poor rural communities and address food insecurity through the provision of meat, milk and other products. A large number of South African rural livestock are challenged by feed insecurity, which leads to livestock starvation and undernourished (Nkosi *et al.*, 2011). The quality and quantity of natural pastures or forages are inadequate, which are often exacerbated by climate change and overgrazing, with livestock productivity most restricted by the low value and forage availability during the dry season and in

drought years (Ngongoni *et al.*, 2006). Moreover, for legumes that can be accessed during dry seasons, palatability by livestock is a problem due to presence of bioactive compounds that limit the intake and degradability of substrates.

Conservation of maize straws can produce silages of high energy but low protein concentration (Ivan *et al.*, 2018; Uher *et al.*, 2019). On the other hand, legumes alone do not ensile well owing to their high moisture content, high buffering capacity, and low water-soluble carbohydrate (WSC) levels, resulting in high effluent losses and unstable silage of high pH (Artabandhu, 2018). The incorporation of legumes in maize stover and other low-quality roughage or straw silage enhances protein concentration and therefore the nutritional quality of the silage (Ngongoni *et al.*, 2007; Amole *et al.*, 2013; Soe Htet *et al.*, 2021). Therefore, there is a need to supplement legumes in maize straws to improve intake and digestibility.

1.3 Justification

In the rural communities, sheep and goats generate wealth as a household source of revenue and prestige in both women and youngsters. One of the main constraints to ruminant production is the dearth of feed particularly in the dry season. It is therefore important that the preservation by ensilage of legumes and maize during the rainy season be a custom for most smallholder sheep owners. Herbaceous forage legumes have been described as possible protein supplements for ruminants, as they contain high CP, vitamins, as well as minerals required to support ruminal microorganisms (Malebana *et al.*, 2018; Washaya *et al.*, 2018). Supplementing maize diets with legumes increases feed intake, digestibility and animal efficiency or performance (Owens *et al.*, 2010). The CP concentration of the legume species such as *Lablab purpureus* and *Vigna unguiculata* exceeds requirements for lactation (120 g/kg CP DM) and growth (113 g/kg DM) in

ruminants (ARC, 1984). In the previous studies (Mokoboki et al., 2002, Ravhuhali *et al.*, 2011), herbaceous legume species contained low concentrations of total phenolics and tannins, and consequently the these bioactive compound levels would not be expected to have toxic effects on ruminants. The inclusion of *Lablab purpureus* and *Vigna unguiculata* legumes, more specifically as a source of protein may aid in augmenting the growth performance of ruminants, particularly during winter. There is dearth of research on the benefits of maize-legume silages for smallholder ruminant production. It can therefore be anticipated that ruminant livestock farmers in South Africa and other parts of developing countries can profit from the use of legume-maize silage to substitute costly commercial protein sources. Preserving high-quality forages thus eliminates the need to buy protein concentrates during drought or winter for use in ruminant production.

1.4 Objective

The broad objective of this study was to determine the effect of supplementing legume leaves in maize straws on nutritive value, palatability indices and silage fermentation parameters

Specific objectives were:

1. To assess the nutritive value and use the components to predict the digestible energy (DE), metabolizable energy (ME), total digestible nutrients (TDN) dry matter intake (DMI), dry matter digestibility (DMD) and the relative feed value (RFV) of legume leaves and maize straw.
2. To assess the palatability indices, intake and *in vitro* ruminal dry matter degradability, legume leaves and maize straws.
3. To assess the possible effect of the inclusion of legume leaves in maize straws silage on the nutritive value, *in vitro* dry matter degradability and fermentation characteristics

1.5 Hypotheses

- i. There will be a variation on the nutritive value, *in vitro* ruminal dry matter degradability, intake, and palatability indices of legume leaves and maize straws.
- ii. The inclusion of legume leaves on maize straws silage will influence nutritive value, *in vitro* ruminal dry matter degradability and fermentation characteristics.

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Chapter 2: Literature review

2.1 Introduction

Small ruminants play a noteworthy role in the livestock industry in South Africa. These animals, however, rely on the grassland as their basic feedstuff. Seasonal variations in both quantity and quality constrain the value of the grassland in both quality and quantity (Baloyi *et al.*, 2008). The low protein content and high fibre content in grassland limit intake and degradability in the rumen, which lead to low animal production (Mokgakane *et al.*, 2021; Msiza *et al.*, 2021). In many tropical and subtropical countries, the low productivity of ruminants is attributed to low digestibility and low nitrogen content of available roughages (Beyene & Mlambo, 2012). Supplementation with high protein legumes is one strategy to reduce under-nutrition (Singh *et al.*, 2006).

It has been reported that the supplementation of low-value roughages with forage legumes in ruminant feedstuffs improves intake and digestibility (Baloyi *et al.*, 2008; Ngongoni *et al.*, 2008; Owens *et al.*, 2010). Ruminant feed resources in tropical regions usually consist of natural pastures and poor-quality grasses that are limited in supply throughout the dry season (Gulwa *et al.*, 2017). Thus, ruminant feeding with preserved feeds such as silage or hay has a potential to be an effective feeding stratagem as it could be made available throughout the year. This chapter reviews goat production and its role in South Africa, characteristics of selected forage legumes as potential fodder crops, silage and principles of ensiling, use of molasses to enhance silage fermentation, effect of the inclusion of legumes in maize straws on silage characteristics, palatability indices, and degradability of substrates.

2.2 Goat production and its role in South Africa

The relatively modest input to total farm revenue and the reduction in livestock numbers especially goats over the previous three decades has led to a decline in the significance of the livestock industry to the nationwide economy (DAFF, 2020). One of the reasons could be the consequences of poor nutrition that results in low productivity (Aw-Hassan *et al.*, 2010), and reduced grazing fields due to mining and urbanization. The livestock industry is located arid regions with a carrying capacity ranging from less than one large stock unit per 40 ha to relatively intensive pasture-cropping and intensive agricultural region enterprises. According to DAFF (2020) and RPO (2020), the coastal regions followed by the central provinces have goat production as the leading livestock sector (Table 2.1). The national distribution of goats is as follows: 1.9 million in the province of Eastern Cape, 902 000 in the province of Limpopo, and 662 000 in the province of the KwaZulu Natal. The remaining total of 1.6 million being shared among the other South African provinces (DAFF, 2020; RPO, 2020).

Goats are valuable resources for rural poor farmers because they help to ensure food security and alleviate poverty (Ng'ambi *et al.*, 2018). Rantlhwatlhwa (2019) and Tsvuura (2020) indicated that goats also provide key protein sources in the form of milk as well as meat. Goat milk is beneficial to those who are allergic to cow's milk or have a weakened immune system (Tsvuura, 2020).

Table 2.1: Provincial ruminant livestock numbers in the RSA

Province	Cattle			Sheep			Goats		
	Aug-19	Nov-19	Aug-20	Aug-19	Nov-19	Aug-20	Aug-19	Nov-19	Aug-20
Thousands									
Eastern Cape	3 082	3 096	3050	6 531	6 549	6 513	2 005	2 003	1991
Free State	2 109	2 114	2054	4 518	4 384	4 330	217	216	215
Gauteng	246	245	246	87	86	84	23	24	21
KwaZulu-Natal	2 481	2 430	2380	656	652	628	680	677	662
Limpopo	898	894	860	204	200	199	902	885	902
Mpumalanga	1 243	1 239	1248	1 554	1 544	1527	79	79	77
North West	1 578	1 576	1576	607	603	596	668	661	651
Northern Cape	433	430	419	5 305	5 292	5 182	469	464	488
Western Cape	488	481	466	2 623	2 603	2 545	207	207	202
Total	12 558	12 505	12 299	22 085	21 913	21 604	5 250	5 216	5 169

Source: DAFF (2020); RPO (2020)

In some parts of the country, due to religious beliefs, goat meat is the first choice (Traoré *et al.*, 2018). Goats are also vital during traditional ceremonies performed by traditional healers for individuals who believe in traditional medicine as well as rites (Betterncourt *et al.*, 2015; Mdletshe, 2015). Furthermore, they provide cash from the sale of their products such as mohair, skin, milk and meat (Brown *et al.*, 2018, Mseleku, 2018; Mohale, 2019). They also provide job opportunities as men are employed as shepherds. In grazing systems, bush encroachment or proliferation of invasive species is a major problem, so the introduction of browsers like goats can help to control (Moela, 2014; Ravhuhali *et al.*, 2021). According to Abdul-Aziz (2010), small stock animals are also significant because of their small size, productivity, and their non-competitiveness with people for food.

2.3 Characteristics of selected forage legumes as potential fodder crops

2.3.1 *Lablab purpureus*

Lablab purpureus (Figure 2.1) is a dual-purpose legume, historically grown in the south also Southeast Asia and Eastern Africa as a pulse crop for human consumption. The legume is also utilized in tropical areas with summer rainfall as a forage legume for foraging and protection in large-acre agricultural systems (Ewansila *et al.*, 2007). According to Musumba (2021), lablab is a short-lived perennial or twinning herbaceous plant with robust trailing. Lablab reacts well to strip grazing where there is removal of stock after consuming the leaves but before grazing the stems too low (Aganga & Tshwenyane, 2003). According to Ayisi *et al.* (2004), germination usually takes place within five days with maturity between 60 and 150 days after planting. Climbing varieties develop up to a metre in height with elongated trunks (up to 5 m) (Al-Snafi, 2017). The fronds are pubescent, trifoliate, 2-15 cm in length, and 1.5-14 cm broad (Heuze *et al.*, 2016; Al-Snafi, 2017)). The dorso lateral aspect of the frond is plain with a hairy underside (Evans, 2002).



Figure 2.1: Lablab legume grown in the North-West University experimental field

Karachi (1997) reported lablab (cv Rongai) DM yield of 76.4 g/plant of leaf and 84 g/plant of stems with 25% and 11.88% CP for leaves and stems, respectively. Aganga & Autlwetse (2000) recorded 16.4 % CP in the entire plant hay, higher than the 12.7-14.1% reported by Evans (2002). Washaya *et al.* (2018) reported mean lablab forage concentration of CP (17% DM), which is higher than the proposed minimum lactation requirements (12 % CP DM) and growth requirements (11.3 % DM) for ruminants (ARC, 1984). Ratnawaty *et al.* (2013) reported mean CP (18.73% DM), ADF (42.08% DM), NDF (61.88% DM), and DM (77.88% DM) as shown in Table 2.2. The variance in legume nutritive value is influenced by frond: trunk ratio (Shehu *et al.*, 2001). Frond: trunk proportions are additionally since the fronds are metabolic organs (Shehu *et al.*, 2001). Karachi (1997) recorded mean frond content of neutral detergent fibre (NDF) (14%) and acid detergent fibre (ADF) (29.6%) that were 20.5 units less than the values for the trunks.

2.3.2 *Vigna unguiculata*

Cowpea (*Vigna unguiculata*) (Figure 2.2) is a yearly herbaceous legume mainly planted for its palatable seed as well as feed (Singh, *et al.*, 2003, Ntombela, 2012). Its growth depends on the variety, it can be climbing and erect, or prostrate and creeping. The climbing varieties can grow up to 2 m while the prostrate varieties can grow up to 0.8 m. The leaves are trifoliate, 6-15 cm long and 11-14 cm wide, with oval leaflets. Cowpea is more drought-tolerant and grows on low fertile soils than any other legume crops (Matsui & Singh, 2003; Timko *et al.*, 2008; Mashilo, 2013).



Figure 2.2: Cowpea (Dr Saunders) legume grown in the North-West University experimental field

Normally, cowpeas have high nutritional value and they do not cause bloating in cattle (Mbukwane, 2017). Cowpea is a rich CP source for livestock ruminants (Iqbal, 2015). Several authors have reported a range from 150-250 g/kg DM CP content in cowpea forage. Ansah *et al.* (2021) reported the CP content (154 g/kg DM) in Padi Tuya variety. Etana *et al.* (2013) found the range of 19.22-22.3% DM for CP in cowpea varieties. Gebreyowhans & Gebremeskel (2014) stated that the CP content found in cowpea leaves is in the range of 28-30% which is more than other parts of cowpea. According to Iqbal (2015), the acid detergent lignin (ADL) is between 10-

13 % DM in cowpeas. The cowpea varieties have neutral and acid detergent fibre (NDF and ADF) content between 41.4-51.4 % DM and 36.7-45.5 % DM respectively (Mbukwane, 2017). The cowpea ash content is normally between 13-15 % DM (Iqbal, 2015).

Table 2.2: Chemical composition of selected tropical legumes (% DM)

Tropical legumes	DM	CP	NDF	ADF	References
<i>Lablab purpureus</i>	77.88	18.73	61.88	42.08	Ratnawaty <i>et al.</i> , 2013
<i>Vigna unguiculata</i>	89.00	16.89	23.00	20.00	Abebe, 2015

2.4 Maize straw

Maize straw (*Zea mays* L.) is a rich energy feed for ruminants, especially when it comprises stalks, leaves and ears. Maize is the world's most vital cereal for both human consumption and feeding livestock. Maize grain is utilized for all animal feeding, whereas the entire maize straw is customarily utilized as silage for ruminants (Aquino *et al.*, 1999). Foraging entire maize straw plants also offer livestock with feedstuff during times of insufficiency (dry and hot summers, and winters). Maize straw is a valued source of feedstuff for smallholder possessed stock in regions where conditions are harsh and feed is scant (Methu *et al.*, 2006). Maize is a high-energy feed, superior to other tropical feeds, with DM frequently digestible lower than 40%.

2.5 Silage and ensiling processes

2.5.1 Silage

Silage is the forage material from green foliage plants preserved to the point of acidification through fermentation (McDonald *et al.*, 1991; Wood, 2012). Preservation of forage by ensiling requires a spontaneous anaerobic fermentation of water-soluble carbohydrates (WSC) by lactic acid bacteria into lactic acid (LA) and, to a lesser degree, acetic acid (AA). The production of acid reduces the pH and as a result inhibits the activity of undesirable microorganisms for instance species *Clostridium* which causes spoilage (McDonald *et al.*, 1991). To ensure ultimate conditions for conservation, the forage material needs to be densely compressed to remove air. Afterwards, successful sealing should preserve anaerobic conditions. The primary aim of silage processing is to optimize the retention of primary nutrients in the forage crop for livestock feeding at a future date (Stewart, 2011).

2.5.2 Silage making process

Silage making is a vital approach for farmers to feed ruminant livestock when natural grassland is not in great condition, for instance in winter. When the legumes and maize reach their maximum nutrient level that is typically just before they are fully grown, they must be cut (Shinoda *et al.*, 1999). It is vital to cut at that point as all types of stored grass, for instance, hay and silage would have lower levels of nutrients than fresh pasture, making the end product as nutritious as possible (Tikwayo, 2016).

The achievement of anaerobic conditions is a vital goal in conserving maize and legumes through natural fermentation. Anaerobiosis in nature can be achieved using different techniques. The best effective option would be to keep the forage material in an airtight closed jar, and under such conditions, the herbage-trapped oxygen would be easily extracted by the plant's respiratory enzymes (Tikwayo, 2016). The primary purpose of the closing is to preclude re-entry and airflow throughout storage. According to Mciteka (2008), if oxygen is in contact with herbage for some time, microbial aerobic action takes place and the forage decomposes into deteriorated, unpalatable, and often poisonous constituent.

Ruminants animals normally prefer the odour resulting from the acids formed throughout ensiling to hay, because it is highly palatable and of higher feed quality. Bacteria on the forage ferment plant sugars during ensiling to form organic acids (for example LA and AA) that lower the pH of the silage till bacteria can no longer propagate. The silage stays conserved provided that air stays out (Kunkle & Chambliss, 2002; Schoeder, 2004).

If LA is the bulk of the acid formed, it is possible to achieve quality silage as it is the greatest competent acid of fermentation and will swiftly decrease the silage pH. The sooner the

fermentation is done, the more nutrients the silage contains. A rise in lactic acid promotes those bacteria that break down simple sugars. The pH drops between 3.8 to 4.2 and acidic conditions together with bacterial activity stabilize at a certain level. According to Kunkle & Chambliss (2002), the ensiling cycle takes many days and can be divided into 4 phases.

Phase 1: This will last a few hours after the silo is sealed. It is the time when aerobic respiration of plant cells and aerobic microorganisms use the atmospheric oxygen trapped within the forage material. Elferink *et al.* (2000) stated that the plant enzymes for instance proteases as well as carbohydrases are active and this will lead to the degradation of complex structural carbohydrates such as hemicellulose. This process is heavily dependent on compaction and sealing efficiency.

Phase 2: As soon as air is used up, the anaerobic bacteria start fermenting plant sugars into lactic, acetic, and other organic acids, alcohols, carbon dioxide, and nitrogen compounds. This process may continue for two to four days during which silage pH is lowered by the organic acids from above 6 to below 5

Phase 3: Unless the anaerobic conditions are retained, there will be no change or little change will occur and the silage stays conserved for a long time. With the pH dropping to or below 4.0, the number of microbes declines. During this process, acid-tolerant microbes live in a dormant state, and *Clostridia* and *Bacilli* exist as spores. This process is limited for forage crops with sugar concentrations lower than 8% of the DM since fermentable sugars are reduced.

Phase 4: This phase begins the minute the storage tower is unlatched to feed the silage to livestock ruminants. Air penetrates the silo and yeasts and moulds are revived which were dormant during fermentation. Fungi then use the sugars, lactic acid, and other nutrients for their growth and yield by-products such as carbon dioxide as well as warmth. Insufficient lactic acid concentration results

in increased decomposition, loss of DM, and decreased silage palatability (Elferink *et al.*, 2000). Additives or inoculants that impede the growth of spoilage yeasts along with moulds can improve bunk life and maintain the silage quality.

2.6 The use of molasses to enhance silage fermentation

In silage production, critical management stages include the ensiling as well as feed out phases to decrease nutrient loss and enhance the nutritional value. To control unwanted microorganisms and enhance aerobic stability in silages, many chemical and biological additives have been used (Pedroso *et al.*, 2011; Kebede *et al.*, 2018). In a study by Lima *et al.* (2010), Nkosi *et al.* (2012) and Ni *et al.* (2017) addition of molasses has been known to enhance silage fermentation thereby increasing LA, decreased AA, resulting to decrease in ammonia and the pH. According to Moran (2005), a 3-4% addition level is needed to reduce the pH of the silage. The addition of molasses also aids in reducing the development of spoilage bacteria (Ni *et al.*, 2017). This occurs when LAB use the fermentable sugars in molasses to thrive and replicate quickly, thus out-competing other bacteria (Babaeinasab *et al.*, 2015; Ni *et al.*, 2019; Musumba, 2021). Luo *et al.* (2021), reported that the addition of molasses to alfalfa resulted in reduced NDF, while CP and ADF remained stable in the silages.

2.7 Effect of inclusion of legumes in low quality roughages on ensiling characteristics and nutritive value

Previous research (Tao *et al.*, 2020) suggested that ensiling characteristics generally control microbial activity by a combination of an anaerobic environment and a natural fermentation of sugars by lactic acid bacteria on legume forages. According to Wang *et al.* (2017), quality silage is

dependent on rapid propagation of LAB that ferments WSC mainly to LA, which reduces silage pH and inhibits the growth of undesirable bacteria, consequently minimizing nutrition losses.

Ensiling maize with *Bauhinia variegata* reduced butyric acid, ammonia-N, and free amino acid levels. Moreover, the bacterial diversity was improved, while the amount of *Clostridium* was decreased and LA producing bacteria was improved (He *et al.*, 2020). Goyal & Tiwana (2016), reported that an increase in levels of cowpea in corn resulted in increased ammonia-N content as well as pH in the silage and thus silage quality was reduced. Carpici (2016) stated that the addition of soybean in maize increased the silage pH values and the pH started to increase steadily after 50% addition. Amuda & Tanko (2019) reported that the inclusion of legumes in maize did not affect legume-maize silage characteristics, for instance, the pH values were similar across all treatments. Some authors have found that the inclusion of legumes in low-quality roughages results in increased CP content in the silages (Goyal & Tiwana, 2016; Wang *et al.*, 2017). Topçu & Özkan (2021) reported that sunn hemp can produce good silage and enhance the nutritive value of low-quality roughage silage. This makes legumes to be the preferred choice in silage making due to their high protein content as opposed to other conventional diets.

2.8 Palatability and forage preferences by goats

In some parts of South Africa, legume forages have been identified as a tactical feed resource for ruminants, capable of meeting ruminant feed requirements during critical periods while not reducing nutrient intake (Seresinhe *et al.*, 2012; Brown *et al.*, 2016). However, ruminants choose their feed based on its palatability, which is a complicated phenomenon influenced by both animal and feed characteristics (taste, smell) (Ngwa *et al.*, 2003; Mokoboki *et al.*, 2011; Rojas-Hernandez *et al.*, 2016). Some studies have reported that the nutritive value of a feed influences its palatability

(Deng *et al.*, 2017; Idan *et al.*, 2020). Other authors have reported that plant factors such as species, growth stage, maturity, morphology and phenology, also have an impact on the palatability of the feed (Obour *et al.*, 2015; Ziblim *et al.*, 2019;). Secondary metabolites such as tannins, alkaloids, glycosides and volatile oils, are other plant factors that affect palatability (Gwanzura *et al.*, 2011; Obour *et al.*, 2015; Rojas-Hernandez *et al.*, 2016; Deng *et al.*, 2017).

Previous research on the impact of palatability on feed intake by ruminants are inconsistent. While feed palatability did not affect intake (Minson & Bray 1986; Bums *et al.*, 1987), palatability caused disparities in intake (Sultan *et al.*, 2009; Brown *et al.*, 2016). There are several contradicting reports concerning the effect of chemical components (CP, ADF, NDF, ADL, CT and phenols) on feed palatability (Obour *et al.*, 2015). Mokoboki *et al.* (2011) and Gwanzura *et al.* (2011) found poor association between chemical components and intake and palatability. In contrast, Brown *et al.* (2016) found a strong association between chemical components and palatability.

2.9 *In vitro* dry matter degradability (DMD) of legume forages

According to McDonald *et al.* (2011), rumen degradability is important in determining feed quality. *In vitro* ruminal DMD has been recently favoured over *in sacco* because it is fast, humane, as well as less labour intensive. Moreover, *in vitro* is used to simplify the *in sacco* by eliminating the need for a large number of live animals (Chaudhry, 2008). The *in vitro* dry matter digestibility of legumes and grasses have been studied. There were differences in the results due to the method used, genotype and environmental conditions (Solomon & Kibrom, 2014). According to Soul (2017), degradability is limited by the plant growth stage. This was ascribed to legume lignification as the legume grows older. In previous studies, cowpea had better digestibility than lablab (Ravhuhali *et al.*, 2010; Mahala *et al.*, 2012). Zhang *et al.* (2012) stated that legumes have quicker degradation when compared to grasses, and the presence of legumes may hasten grass degradation.

2.10 Summary

Small ruminants play a role in the livestock industry in South Africa and other developing countries and these ruminants are challenged by feed insecurity, which lead to low animal production. The nutritive value of grasses varies seasonally in both quantity and quality. The expectations are that there is low protein content and high fibre content in grassland during dry season that limit intake and degradability in the rumen. Moreover, high protein and palatable legumes can be accessed during dry seasons. Supplementing low quality diets such as maize straw with legumes increases feed intake, digestibility and animal performance. Preserving high-quality forages as silage can eliminate the need to buy protein concentrates during drought or winter for use in livestock industry. These legumes can be challenging due to presence of anti-nutritional factors that limit the intake and degradability of substrates. Therefore, it can be anticipated that ruminant livestock farmers in South Africa and other parts of developing countries can profit from the use of legume-maize silage to substitute costly commercial protein sources.

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Chapter 3: Chemical component prediction from nutritive value of legume leaves and maize straws

Abstract

The objective of this study was to assess the chemical composition and use the components to predict the digestible energy (DE), metabolizable energy (ME), total digestible nutrients (TDN) dry matter intake (DMI), dry matter digestibility (DMD) and the relative feed value (RFV) of legume leaves and maize straw. Four legume species, *Lablab purpureus*, *Vigna unguiculata* (Dr Saunders, Betswit, and indigenous cowpea varieties) were planted in monoculture in the experimental field, replicated four times. Maize (*Zea mays*) was similarly planted in a monoculture. The legume leaves and maize straw were analyzed for chemical composition. All data were subjected to a one-way analysis of variance (SAS, 2010) in a completely randomized design. Maize straw had the highest ($P<0.05$) dry matter (DM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) content when compared to other forages. The highest ($P<0.05$) CP content was recorded for the Dr Saunders (230.7 g/kg DM). Betswit had the highest ($P<0.05$) ash, ether extract (EE), acid detergent lignin (ADL) content and condensed tannins (CT) concentration among all the forages. Lablab (0.36 g/kg DM) had a higher ($P<0.05$) total phenols than all forages. Lablab and the indigenous cowpea had the highest ($P<0.05$) ME content. Highest ($P<0.05$) RFV was recorded for lablab. Lablab and indigenous cowpea had the highest chemically predicted DE value (2.77 and 2.76 Mcal/kg). Lablab, indigenous cowpea, and Dr Saunders had better ($P<0.05$) CP, CT and the predicted DE and ME values. In conclusion, the chemical and predicted composition of all the test legumes was considered adequate for supplementation to low-quality maize straw.

Key words: nutritive value, legumes, ruminants, digestibility, low-quality roughages, supplement

3.1 Introduction

Animal products are in higher demand in southern Africa due to increasing population, urbanization and incomes (Malebana *et al.*, 2018). However, animal production is restricted due to the quantitative and qualitative feed deficit, particularly during the dry periods. Several authors (Mnisi & Mlambo, 2017; Ramantsi *et al.*, 2019; Nkosi *et al.*, 2019) highlighted that during dry periods, available pastures are fibrous with low crude protein (CP) which is the main restricting nutrient. For optimal animal production, the available pastures during this period must be supplemented with a cheap protein source such as legumes (Rusdy *et al.*, 2019). Legume leaves can be accumulated in large amounts and incorporated in ruminants' diets due to their high feeding value when compared to grasses that normally deteriorate during the dry periods. This supplementation aids in better utilization of low-quality pastures by providing nitrogen to rumen microflora (Nkosi *et al.*, 2019).

According to Daba *et al.* (2019), proper chemical characterization of legumes facilitates efficient feeding to ensure ruminant productivity. Moreover, prediction of nutritive indices from chemical components is also used to evaluate forage quality. The most frequently used parameters to evaluate forage quality are CP, metabolizable energy (ME) and total digestible nutrients (TDN) (Amiri & Mohamed Sherrif, 2012). According to Mut *et al.* (2010), relative feed value (RFV) can also be used, as it indicates the palatability and digestibility of forage. There is a need to continue screening the forage available to meet the animal demands. Therefore, the objective of this study was to assess nutritive value and predict chemical estimates from chemical composition parameters of legume residue and maize straw.

3.2 Material and methods

3.2.1 Study site

This study was conducted at the North-West University farm (Campus), Mafikeng (25°49'22" S and 25°36'54" E), North-West province of South Africa, with an altitude of about 1290 m over sea level. The prevailing temperature varies from 27 to 38 °C throughout summer and between 11 °C and 18 °C throughout the winter season. The area gets an average yearly rainfall ranging from 200 to 450 mm yearly and most of the rainfall is usual throughout the summer season.

3.2.2 Legume planting

Four legumes species were used in the experiment which were grown under similar soil and management conditions. The soil type from the planting site resembles those of Clovelly and Hutton form. Four legume species, *Lablab purpureus*, *Vigna unguiculata* (Dr Saunders, Betswit, and indigenous cowpea varieties) were planted in monoculture in the experimental field, replicated four times. They were assigned to the plots in relation to a randomized complete block design (RCBD). Each block had 4 plots (size of 13m x 7 m each plot) for each of the 4 different legumes thus yielding 16 plots. The legumes were sowed in rows with inter-row spacing of 30 cm. On the day of planting, all legume seeds were inoculated with the appropriate *Rhizobium* strains to make full use of the nitrogen fixation advantages. The rows were oriented east-west to minimize the retardation of legume growth because of shading by the high-growing cereal crops since the shade should fall within the row. Maize was sowed in rows with an inter-row spacing of 90 cm as well as an intra-row spacing of 17 cm in an area of 20 m x 25 m.

3.2.3 Forage management and harvesting

The weeding was done when weeds started to germinate. Throughout the growth phase, the crops were inspected frequently for signs of infection as well as pests, and remedial action was taken

where necessary. The sprinkler was used to water plants in the morning, three times a week. Harvesting was done when maize was at the soft dough stage and the legume at the flowering stage (13 weeks old). The legume leaves and maize straw were harvested from the plots respectively and were left to dry at room temperature for two weeks before grinding. The samples were ground in Wiley Mill to pass through a 1-mm sieve and placed in an airtight containers pending chemical analysis.

3.2.4 Chemical analyses

The samples were chemically analysed at North-West University experimental farm (Molelwane), Mafikeng (25°47'27" S and 25°37'18" E). One (1) gram of every sample was weighed into pre-weighed crucibles and put in an oven for 12 hrs at 105 °C to determine dry matter (DM). After weighing DM, samples were placed in muffle furnace for 6 hrs at 550°C to measure ash (AOAC, 2012; method number 973.18). Neutral detergent fibre (NDF), as well as acid detergent fibre (ADF), were determined by the ANKOM²⁰⁰⁰ Fibre Analyser (ANKOM Technology, New York). Acid detergent lignin (ADL) was determined utilizing H₂SO₄ (72%) for 3 hrs as stated by van Soest *et al.* (1991). Total nitrogen (N) was determined by the standard macro Kjeldahl method (AOAC, 2012; method number 976.06) and was converted to crude protein (CP) by multiplying the % N content by a factor of 6.25. Ether extract (EE) was determined using the Soxhlet method (AOAC, 2012; method number 920.39). Hemicellulose and cellulose were calculated: hemicellulose =NDF-ADF, cellulose=ADF-ADL. Total phenolics were determined using Folin-Ciocalteu methods and expressed as tannic acid equivalent (g/kg DM) as stated by Waterman and Mole (1994). Condensed tannins (CT) were determined using Butanol-HCL method and expressed as leucocyanidin equivalent (g/kg DM) as stated by Porter *et al.* (1986).

The following formulas were used to calculate chemical estimates from chemical composition:

Total digestible nutrients (TDN): $TDN = 82.38 - (0.7515 \times \%ADF)$ (Bath & Marble, 1989).

Dry matter digestibility (DMD)%: $DMD\% = 88.9 - (0.779 \times \%ADF)$ as outlined by Mokoboki *et al.* (2019).

Dry matter intake (DMI): $DMI\% = \frac{1.2 \times \text{body weight}}{\%NDF}$ (Mertens, 2002)

Where body weight is equal to an estimated livestock unit metabolic weight (450 kg).

Relative feed value (RFV): $RFV = \frac{\%DMD \times \%DMI}{1.29}$ (Jeranyama & Garcia, 2004).

Digestible energy (DE): $DE \text{ (Mcal/kg)} = 0.27 + 0.0428 (\%DMD)$ (Fonnesbeck *et al.*, 1984).

Metabolizable energy (ME): $ME \text{ (Mcal/kg)} = 0.281 \times DE$ (Khalil *et al.*, 1986).

3.3 Statistical analysis

Data on chemical composition and chemical estimates was subjected to a one-way analysis of variance (SAS, 2010) in a completely randomized design. The following general linear model (GLM) procedure was used:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where; Y_{ij} - The response variable, μ - The overall mean, D_i - Treatment effect, E_{ij} - Random error

For a statistical test, significance was declared at $P < 0.05$. The least-squares means were compared using the probability of difference (pdiff) option.

3.4 Results

Results of the chemical composition of legumes and maize straw are presented in Table 3.1. The chemicals composition varied significantly ($P < 0.05$) among the forages. Maize straw had the highest ($P < 0.05$) DM content when compared to lablab, betswit, indigenous cowpea as well as Dr

Saunders. Betswit had the highest ($P<0.05$) ash content (169.6 g/kg DM) while lablab and indigenous cowpea had the lowest ($P<0.05$) ash content (101.8 and 103.1 g/kg DM respectively) which did not differ ($P>0.05$) significantly from one another. Dr Saunders had the highest ($P<0.05$) CP content (230.7 g/kg DM) while maize had the lowest ($P<0.05$) CP content (70.6 g/kg DM). The highest ($P<0.05$) EE content (34.5 g/kg DM) was recorded for Betswit.

Table 3. 1: Chemical composition of legumes and maize straw (g/kg DM)

Species	DM	Ash	CP	EE
Maize	915.2 ^a	164.2 ^b	70.6 ^e	19.7 ^d
Dr Saunders	896.8 ^c	136.8 ^c	230.7 ^a	19.5 ^d
Lablab	906.5 ^b	101.8 ^d	222.3 ^b	16.7 ^c
Indigenous cowpea	894.2 ^c	103.1 ^d	152.8 ^c	17.0 ^c
Betswit	896.5 ^c	169.6 ^a	180.7 ^d	34.5 ^a
SE	1.49	1.50	1.04	0.85
P value	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c,d,e} Means in the same column with different superscripts are different ($P<0.05$), DM: dry matter, EE: ether extract, CP: crude protein, SE: standard error

Data of fibre fractions of legume leaves and maize straws is presented in Table 3.2. Maize straw had the highest ($P<0.05$) NDF content (622.6 g/kg DM) when compared to Dr Sunders (352.0 g/kg DM), lablab (353.5 g/kg DM), indigenous cowpea (387.1 g/kg DM) and betswit (394.1 g/kg DM). Maize had the highest ($P<0.05$) ADF content (387.6 g/kg DM) and indigenous cowpea and lablab had the lowest ADF contents (307.3 and 302.7 g/kg DM respectively) which were not significantly

different ($P>0.05$) from one another. Betswit had the highest ($P>0.05$) ADL content while indigenous cowpea had the least ($P<0.05$) ADL content. Maize had the highest ($P<0.05$) cellulose and hemicellulose content while Dr Saunders had the lowest ($P<0.05$) content.

Table 3. 2: Fibre fractions of legume leaves and maize straw (g/kg DM)

Species	NDF	ADF	ADL	Cel	Hemi
Maize	622.6 ^a	387.6 ^a	141.2 ^c	246.4 ^a	235.0 ^a
Dr Saunders	352.0 ^c	316.4 ^c	159.6 ^b	156.8 ^e	35.6 ^d
Lablab	353.5 ^c	302.7 ^d	126.3 ^d	176.4 ^c	50.8 ^c
Indigenous cowpea	387.1 ^b	307.3 ^d	116.7 ^e	190.6 ^b	79.8 ^b
Betswit	394.1 ^b	340.3 ^b	176.7 ^a	163.6 ^d	53.8 ^c
SE	2.55	2.43	2.49	2.16	3.63
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c,d,e} Means in the same column with different superscripts are different ($P<0.05$), NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, Cel: cellulose, Hemi: hemicellulose, SE: standard error

Figure 3.1 shows that betswit ($M \pm SE = 50.1 \pm 2.52$ g/kg DM) had highest CT concentration ($P<0.05$) than lablab (28.0 ± 2.52 g/kg DM), indigenous cowpea (28.0 ± 2.52 g/kg DM), Dr Saunders (24.0 ± 2.52 g/kg DM) and maize (0.06 ± 2.52 g/kg DM).

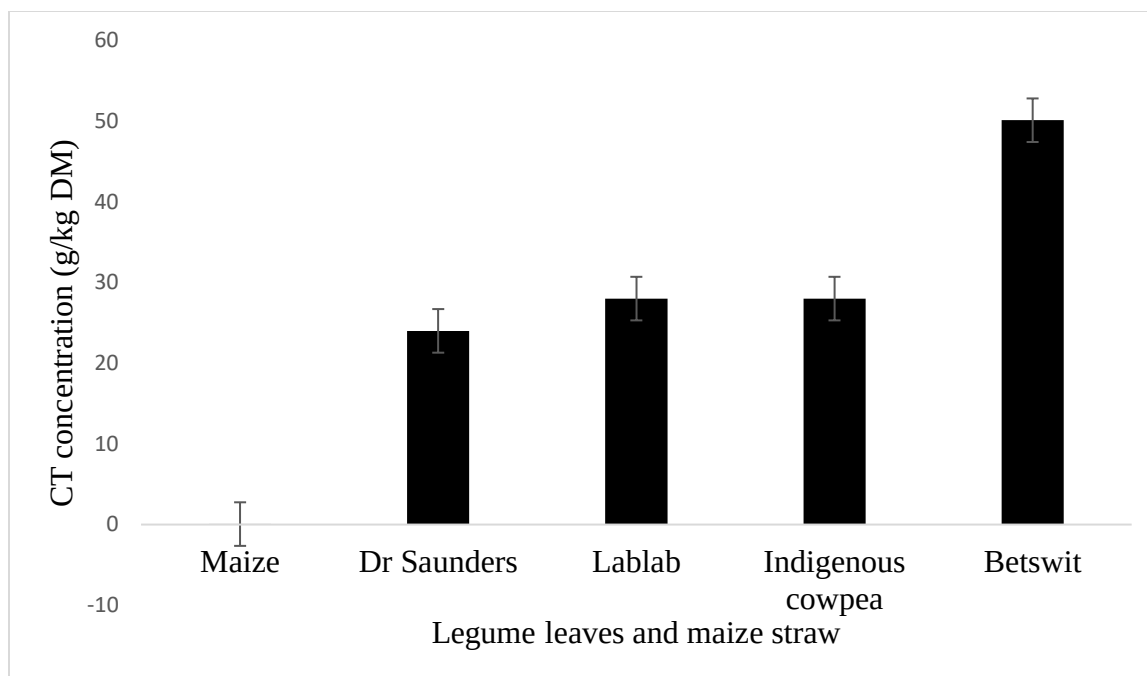


Figure 3. 1: Condensed tannins concentration of legume leaves and maize straw

Figure 3.2 shows that lablab ($M \pm SE = 0.36 \pm 0.02$ g/kg DM) had higher ($P < 0.05$) total phenols than Dr Saunders (0.23 ± 0.02 g/kg DM), maize (0.20 ± 0.02 g/kg DM), indigenous cowpea (0.17 ± 0.02 g/kg DM) and Betswit (0.10 ± 0.02 g/kg DM).

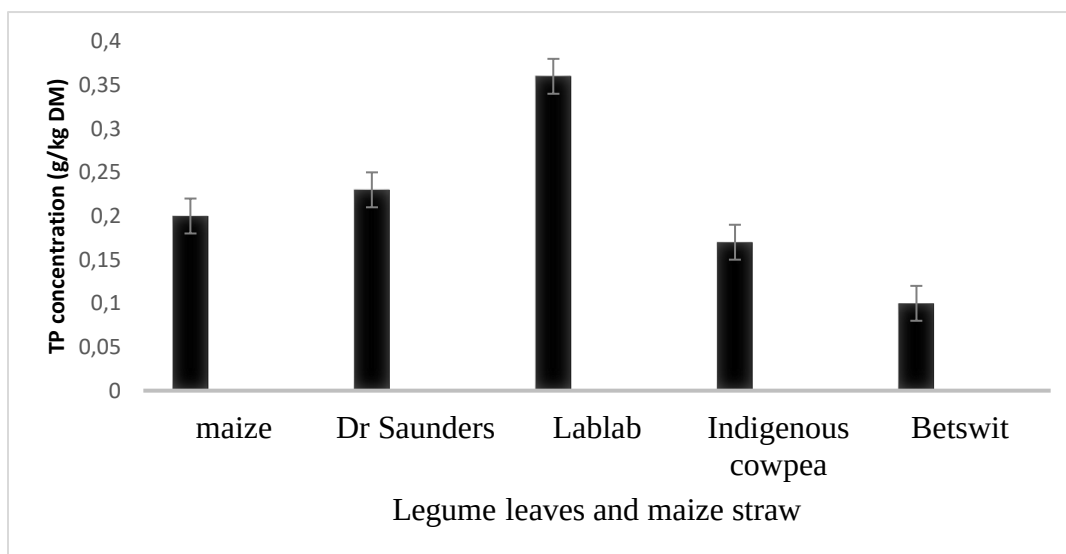


Figure 3. 2: Total phenolics of legume leaves and maize straw

Results of chemical estimates from the chemical composition are presented in Table 3.3. Highest (P <0.05) DMD, TDN, DE, and ME values were recorded for lablab and indigenous cowpea. Lablab and Dr Saunders had the highest (P<0.05) RFV value than all the forages. The lowest (P<0.05) DMI value was recorded for maize when compared to other forages.

Table 3. 3: Chemical estimates from chemical of legume leaves and maize straw

Species	DMD	RFV	DMI	TDN	DE	ME
	(%)		(%)	(%)	(Mcal/kg)	(Mcal/kg)
Maize	50.1 ^d	337.1 ^d	8.8 ^c	53.2 ^d	2.41 ^d	2.01 ^d
Dr Saunders	57.3 ^b	680.9 ^a	15.3 ^a	58.6 ^b	2.72 ^b	2.26 ^b
Lablab	58.6 ^a	694.3 ^a	15.3 ^a	59.6 ^a	2.77 ^a	2.31 ^a
Indigenous cowpea	58.2 ^a	629.1 ^b	13.9 ^b	59.3 ^a	2.76 ^a	2.29 ^a
Betswit	54.7 ^c	582.7 ^c	13.7 ^b	56.8 ^c	2.61 ^c	2.18 ^c
SE	0.24	4.63	0.08	0.18	0.01	0.01
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c,d,e} Means in the same column with different superscripts are different (P<0.05), DMD: dry matter digestibility, RFV: relative feed value, DMI: dry matter intake, TDN: total digestible nutrients, DE: digestible energy, ME: metabolizable energy, SE: standard error.

3.5 Discussion

Dry matter (DM) refers to the amount of feed that remains after moisture have been removed (Abdullah *et al.*, 2017). The findings of the current study showed that the DM content was high in the forages. The DM content of maize was higher when compared to all legumes. The current results of maize showed similar results to the results of Nwadike & Sali (2017). According to Kokkonen *et al.* (2000), DM content is vital as it determines the quantity of nutrients required for formulating feeds for intake, which subsequently has an effect on animal production.

The concentration of ash is directly linked to the high proportion of minerals such as calcium, phosphorous, potassium and the large fraction of silica (Rambau *et al.*, 2016). Minerals are vital for animal health (Msiza *et al.*, 2021). The ash content of maize in the present study was higher than the value obtained by Methu *et al.* (2001). The ash content obtained in this study was similar to the ash content reported by Gebreyowhans & Gebremeskel (2014) for cowpeas. Ash content of lablab was 101.77 g/kg DM, this value falls between the range 8.6-10.8% obtained by Musumba (2021), but lower than the 12.52% reported by Mbahi *et al.* (2018). The slight variations might be due to different amounts of rainfall, leaf shedding, harvesting stage, as well as soil fertility (Mbahi *et al.*, 2018).

Ether extracts (EE) contains crude fat, which is made up of all ether soluble substrates. It contains lipids, which provides energy to animals (Mciteka, 2008). Nonetheless, high EE content in a forage reduces forage intake by ruminants and disrupt rumen function (Deng *et al.*, 2017; Muya *et al.*, 2020). In the current study, the EE content (19.5 g/kg) of Dr Saunders was comparable to the content (25 g/kg) obtained by Straub *et al.* (2019) from cowpea leaves. The EE content (34.5 g/kg) of betswit was higher than the 25 and 26 g/kg reported by Straub *et al.* (2019) and Sow *et al.* (2020)

respectively. Ether extract (EE) content (16.7 g/kg) of lablab was comparable to the value (18.9 g/kg) reported by Mbahi & Goska (2017) whereas Babayemi *et al.* (2006) reported a value of 28.3 g/kg. Genetic variations might be responsible for the variations among the forages due to their adaptation to a wide range of environmental conditions (Musumba, 2021). The presence of EE explains the high-energy content (Nkosi *et al.*, 2019), indicating that they can be used as an energy source in feedstuffs. However, the high EE content of Betswit might limit its intake and degradability due to feed particles that are normally covered by EE which lead to the prevention of microorganisms from attacking the particles (Muya *et al.*, 2020).

The nitrogen content determines the crude protein (CP) in a forage. Daba *et al.* (2019) mentioned the importance of CP in forages for ruminants in terms of growth, development and reproduction. All the legumes in the current study had CP of more than 150 g/kg DM; these values were similar to other studies (Mbukwane, 2017; Bhardwaj & Hamama, 2019; Wangila *et al.*, 2021). The CP content of these legumes is in excess of the minimum 12% required for growth and performance of sheep, goats and cattle (Soliva *et al.*, 2015; Tulu *et al.*, 2018). Maize had the lowest CP content (70 g/kg) and this value may not satisfactorily support ruminal microflora activity, thus restraining the content of protein available to the ruminants (Nkosi *et al.*, 2019). The high CP content in legumes used in this study suggest that the legumes can be incorporated into ruminants' diets as a protein supplement to low-quality roughages such as maize straws and grasses. However, CP content of pastures beyond 200 g/kg DM surpasses the CP requirements for ruminants (Pacheco & Waghorn, 2008). This suggests that lablab and Dr Saunders cannot be utilized as grazing forage since the CP content exceed the recommended content by (Pacheco & Waghorn, 2008; Soul, 2017). Surplus CP content can have a detrimental effect on ruminant production since there are substantial metabolic costs associated with disposing of surplus nitrogen (Pacheco & Waghorn,

2008). Soul (2017) explained that extra protein is converted to nitrous oxide, and has an adverse influence on climate change thereby damaging vegetation, decreasing plant growth and yields, and the scarcity of vegetation may limit animal production. There is a need to balance the CP content when lablab and Dr Saunders are included in animal diets.

Acid detergent fibre (ADF) represents essentially the crude lignin, cellulose fractions of plant material and silica (Obregon-Cano *et al.*, 2019). Amary (2016) stated that neutral detergent fibre (NDF) is a depiction of a forage plant's cell composition (pectin, organic acids, carbohydrates and protein). Fibre fractions are significant as they relate to feed intake and digestibility by ruminants (Msiza *et al.*, 2021). The NDF content of cowpeas in this study was comparable to that reported by Tekle & Gebru (2018) while the ADF was similar to that reported by Amare & Girmay (2020). The ADL content of cowpeas was higher than the ADL reported by Gebreyowhans & Gebremeskel (2014). The ADF and NDF content of lablab in this study were significantly lower than the ones reported by Seid & Animut (2018) as well as Jabessa *et al.* (2021). The ADL of lablab in the current study was significantly higher than the content reported by Hidosa *et al.* (2018). In the current study, Betswit had high ADF and ADL, suggesting that it might be difficult to degrade. This is because ADF is primarily composed of cellulose and lignin, both of which are not easily degradable (Washaya *et al.*, 2018). Roy *et al.* (2019) mentioned that feeds with high lignin typically hamper fibre availability to animals. Lablab can be the best legume for livestock due to its low ADF content since lower ADF content in feedstuff enhance substrates digestibility (Ilknur *et al.*, 2012; Washaya *et al.*, 2018). The NDF content (622.6 g/kg DM) of maize was within the range of 57.44 and 65.16% DM reported by Subrahmanya *et al.* (2017). The ADF content (387.6 g/kg DM) of maize was comparable to the 390 g/kg DM stated by Estrada-Flores *et al.* (2006). For grazing animals, the NDF content for forage should be between 30-40 % DM (Hoffman *et al.*,

2001; Van Saun, 2016). The NDF of the legumes in the current study was below 47% (average forage intake) and for maize straws it was higher than 61% (low forage intake) (Msiza *et al.*, 2021). The ADL content of maize in the present study was similar to that obtained by Akinfemi *et al.* (2009). The high fibre content in maize straw in the current study may decrease digestibility or degradability as stated by Rojas-Hernandez *et al.* (2016). This discrepancy between forages from this study to other studies could be ascribed to differing environmental conditions, planting season, maturity and reaping time (Mbahi *et al.*, 2018).

Forage legumes are known to contain bioactive compounds such as tannins and phenols, which may hamper with intake and digestibility or degradability of substrates in the rumen, thereby affecting animal performance (Mokoboki *et al.*, 2002; Washaya *et al.*, 2018, Mudau *et al.*, 2021). In the current study, total phenolics concentrations values were ranging from 0.17 to 0.36 g/kg DM and these values were lower than the values reported by Mokoboki *et al.* (2002) and Washaya *et al.* (2018) for lablab and cowpeas. This suggests that the total phenolics concentrations in the current study would not be anticipated to have negative effects on ruminants (Mokoboki *et al.*, 2002).

The presence of tannins in a forage has the potential to decrease the nutritive value of forage. With exception of Betswit, all legumes used in this study had lower condensed tannins (CTs) (20–40 g/kg DM), and this concentration level is considered to be beneficial as it has an ability to reduce the risks of bloats, increase amino acid absorption and suppress gastrointestinal parasites (Mahwasane, 2018; Sebolai, 2018). Betswit had CT content of 50.1 g/kg DM, which many reporters mentioned that CT content of this value may have detrimental impact on ruminants by reducing forage palatability, intake, digestibility and nitrogen retention (Wang *et al.*, 2018; Washaya *et al.*, 2018). High CT content in forages have been shown to bind to proteins forming

insoluble tannin-protein complexes (Mnisi & Mlambo, 2017), thus, the use of Betswit as a forage for feeding ruminants might be limited by high content of CT (Mahlake & Mnisi, 2020).

The nutritive value of forage determines dry matter digestibility and intake (DMD and DMI) by ruminants which affect animal performance (Kiraz, 2011; Shetty *et al.*, 2017). The DMD% of legumes in the current study was within the range of 50.6 and 70.2% reported by Kaithwas *et al.* (2020) for legumes. The lower DMD% of maize than legumes was associated with high NDF and ADF (Kazemi *et al.*, 2012). According to Yucel (2019), high DMI% values are preferred in predicting the quality of forage, and this is direct link with low fibre concentration of the diet. The DMD% of the legumes was above 50% considered satisfactory for the maintenance of ruminants, particularly pregnant cows (Ganzolez-Hernandez & Silva-Pando, 1999).

Total digestible nutrients (TDN) is usually used to predict the quality of forage (Gursoy *et al.*, 2021). Moreover, it also represents the total amount of digestible nutrients in the forage. Legumes had better TDN values than maize straws in the present study. These results are in agreement with that reported by Aug *et al.* (2019) who mentioned that legumes have higher TDN than non-legumes (e.g. grass, maize straws). Based on the current findings, these legumes can be fed to mature ruminants. The TDN of all legumes was higher than the 44% required for maintaining lactating cows. The TDN of legumes was more than 53% (late gestation) and 53-66 % (lactating) for maintenance of does (NRC, 2007).

The relative feed value (RFV) is a metric that allows for comparison of forage based on intake and digestibility (Mut *et al.*, 2010; Kazemi *et al.*, 2012; Celiktaş *et al.*, 2020). Pang *et al.* (2019) and Kaithwas *et al.* (2020) highlighted that legume forages generally have higher RFV values than non-legumes. Likewise, all the legumes had higher RFV values than maize straw in the present study. This indicates that these legumes are good forage, and would be more palatable and

digestible. Based on the findings of the current study, these legumes can be fed to the best highly productive cows since the RFV values than higher 151 (high quality) (Kazemi *et al.*, 2012; Malinowska & Jankowski, 2021).

According to Mudau *et al.* (2021), digestible energy (DE) is a measure of how much energy is in forage for the ruminants to use. It is considered the second most restrictive aspect after CP in most forages. The DE content in the present study ranges between 2.41 and 2.77 Mcal/kg in the forages. According to Moore & Jung (2001), lignin is important, as it is associated with DE of livestock diets. Indeed, based on the current findings Betswit had higher lignin than other legumes, and lower DE content. Betswit may compromise the productivity of ruminants due to its low DE. Lignin inhibits the digestion of cell wall polysaccharides by acting as a barrier to microbial enzymes, thus having a direct impact on the DE value of a forage (Getachew *et al.*, 2018).

Ruminant forages are made up of several components (e.g. carbohydrates, fats), which are metabolizable to offer energy. In the current study metabolizable energy (ME) of the forages ranged between 2.01 and 2.31 Mcal/kg with the lowest being for maize straws and highest for lablab. Low energy density forages are typically fibrous and utilized inefficiently (Hughes *et al.*, 2012). The ME content of the forages is within the proposed range (0.57-2.78 Mcal/kg) for the maintenance of goats with low or medium activity (Mudau *et al.*, 2021). The finding of this study suggests that these forages might maintain even high activity ruminants such as lactating dairy animals (NRC, 2007).

3.6 Conclusion

Lablab, indigenous cowpea, and Dr Saunders had better CP, CT and predicted DE and ME values. Based on the findings from measured parameters, due high CP and energy, these legumes can be

incorporated in ruminant's diets to supplement low-quality roughages such as maize straws and grasses.

3.7 References

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Chapter 4: Relative palatability, intake and *in vitro* dry matter degradability of legume leaves and maize straws for ruminants

Abstract

Legumes leaves are considered important protein supplements to cereal straws for ruminants. The objective of the study was to determine the effect of legume leaves and maize straws on relative palatability, intake and *in vitro* dry matter degradability (IVDMD). For the palatability study, each of five male boer goats was provided with four feeding troughs, with four different feeds (maize straws and 3 legumes (*Lablab purpureus* - lablab and 2 *Vigna unguiculata* - cowpeas - Dr Saunders, and Betswit). Feeds were used as treatments and goats as replicates in a completely randomized design. Data were subjected to a one-way analysis of variance (SAS, 2010) in a completely randomized design. Previous data for chemical composition in Chapter 3 was used to establish the relationship between palatability and intake. For IVDMD, the rumen fluid was collected from a fistulated Bonsmara cow. Lablab had a higher palatability index and intake ($P<0.05$) when compared to other diets. Intake ($r^2=0.955$) and palatability index ($r^2=0.971$) were positively and significantly ($P<0.05$) predicted from total phenolics. The highest ($P<0.05$) *in vitro* dry matter degradability was recorded for lablab at 24 up to 72hrs. Except for total phenolics, all chemical composition parameters poorly predicted intake and palatability index. Lablab can be highly recommended to supplement low-quality roughages such as grass and straws due to high degradability and palatability indices.

Keywords: degradability, goats, intake, legumes, palatability, regression.

4.1 Introduction

The utilization of legumes as ruminant feed is gradually becoming vital especially in the tropics where crop leaves, as well as natural pastures, are of low quality (Abdulrazak *et al.*, 2001).

Legumes leaves have potential as protein supplements for livestock e.g. goats, sheep as well as cattle fed low protein roughages. However, their preference by ruminants may be influenced by the acceptability and palatability (Obour *et al.*, 2015). The palatability of legumes is a significant factor in the production of goats, especially if the legumes are consumed to provide a main part of the daily nutrient intake (Deng *et al.*, 2017). Chemical components such as phenolics, alkaloids, tannins, and aromatic compounds and other nutrients such as crude protein (CP) and lignin content of forage have been shown to affect forage's palatability and intake (Ngwa *et al.*, 2003; Sandoval-Castro *et al.*, 2005; Deng *et al.*, 2017; Idan *et al.*, 2020). Mokoboki *et al.* (2011) stated that the palatability of a legume depends on its ability to stimulate an animal's organoleptic senses such as texture, taste as well as odour.

Various techniques such as *in situ*, *in vivo*, as well as *in vitro*, are used to assess the nutrient availability in livestock ruminant feed (Nkosi *et al.*, 2019). *In vitro* dry matter recently has been favoured since it is fast, humane, less labour intensive and it eliminates the need for a large number of live animals (Chaudhry, 2008). As the forage mature, the CP decreases (Grev *et al.*, 2020) and the fibre content increases (Habermann *et al.*, 2019), which negatively influencing forage intake (Harper & McNeill, 2015), palatability (Mpanza, 2015), and digestibility or degradability (Dumont *et al.*, 2015). Moreover, the proportion and extent of lignification of cell walls affects degradation in the rumen (Mpanza, 2015), whereby plant species with high lignin usually have low degradability values (Lancaster & Larson, 2022). The low degradability of plant species high in fibre as well as tannins is caused by the decreased penetrative capacity to rumen microorganisms through lignified plant cells as well as tannins' precipitation influence on microorganisms and enzymes (Mahwasane, 2018). The objective of the study was to assess the *in vitro* dry matter degradability (IVDMD), intake and relative palatability indices of legume leaves (Lablab

purpureus and *Vigna unguiculata*) and maize straw (*Zea mays*), while hypothesizing that legumes and maize straws will affect IVDMD, intake, and relative palatability indices.

4.2 Method and Materials

4.2.1 Study site

This study was conducted at the North-West University experimental farm (Molelwane), Mafikeng (25°47'27" S and 25°37'18" E), North-West province of South Africa, with an altitude of about 1290 m over sea level. The prevailing temperature varies from 27 to 38 °C throughout summer and between 11 °C and 18 °C throughout the winter season. The area gets an average yearly rainfall ranging from 200 to 450 mm yearly and most of the rainfall is usually during the summer season.

4.2.2 Chemical analyses

Chemical analysed were as described in section 3.2.4

4.2.3 Palatability trial

4.2.3.1 Dietary treatments

All the forages (maize straw, lablab, Betswit and Dr Saunders leaves) were harvested from the plots as stated in section 3.2.2 respectively and were left to dry at room temperature for two weeks.

4.2.3.2 Animals, house, experimental design, and feeding.

Five castrated male boer goats (weighing $20.4 \pm 3\text{kg}$) were used in this part of the study. The goats were housed in a roofed and half-walled shed with individual feeding pens (1.7 m² length). Each goat was provided with four feeding troughs, each containing maize straw, lablab, Dr Saunders or Betswit leaves. Feeds were used as treatments and goats as replicates in a completely randomized design. The goats had been treated against internal parasites at the initiation of the feeding trial.

Animals were allowed to adapt to the feeds and the environment for 7 days before the commencement of a trial that lasted 5 days, during which the feed was adjusted daily to meet approximately 10% refusals. Throughout the data collection period, each goat was offered 0.5 kg of each feed daily at 08h00. The placement order of feeds in the troughs was randomized daily to prevent habit reflex. Animals were given clean water *ad libitum*.

4.2.3.3 Measurements and calculations

The feed offered as well as refusals were recorded daily for every goat and samples were preserved distinctly according to species and goat. All calculations were done on a DM basis. The following variables were estimated:

T1- average daily intake of maize straw

Ti- average daily intake of the other feeds, where

i = 2, 3, 4, representing Lablab, Dr Saunders, Betswit

A1- the amount of maize straw offered

Ai- the amount of other feeds offered as shown above

According to Kaitho *et al.* (1996), the relative palatability indices (P1 and Pi), which described palatability of individual feeds were calculated as:

$P1 = (T1/A1)/(T1/A1)$ for maize straw

$Pi = (Ti/Ai)/(T1/A1)$ where I = 2, 3, or 4

In this way, Ri was calculated for each feed daily for the period of data collection.

4.2.4 *In vitro* dry matter degradability (IVDMD)

The IVDMD of the samples was determined using the ANKOM Daisy¹¹ Incubator comprising of a thermostatic chamber (39 °C) with four circling jars according to ANKOM (2005). The samples (0.45-0.5 g) were weighed into ANKOM F57 filter bags, and were heat-sealed and placed in the digestion jars. Buffer solutions (2) were prepared and combined in a ratio of 1:5 and 1600 ml of the combined buffers were added to every jar and was warmed to 39 °C. Rumen fluid was collected from a fistulated Bonsmara cow (~550 kg). The cow was on a mixed diet of blue buffalo grass as well as lucerne. Rumen fluid was collected into two pre-warmed thermos flasks and promptly transported to the laboratory, where it was mixed and strained through two layers of warm muslin cloth. Rumen inoculum (400 ml) was added to every jar which contained ANKOM buffer (1600 ml) and F57 bags. To maintain anaerobic conditions, jars were continually purged with CO₂ gas, and incubation was performed at 39 °C. The F57 filter bags were withdrawn after 6, 12, 24, 36, 48 as well as 72 h of incubation. At 0 hr, samples were just washed with distilled water for 20 minutes and during other withdrawal periods, the bags were washed with distilled water for 20 minutes using ANKOM²⁰⁰⁰ Fiber Analyzer, the bags were then desiccated for 12 h at 105 °C earlier having been weighed for IVDMD determination. *In vitro* DM degradability was calculated using this formula:

$$\%IVDMD = \frac{100 - (W3 - (W1 \times C1))}{W2 \times DM} \times 100$$

Where, W1-bag tare weight, W2-sample weight, W3-final bag weight after *in vitro* treatment, C1-blank bag correlation factor (final over dried weight / original blank weight).

For IVDMD and palatability trial, the cow and the goats were cared for according to the guidelines of the Institution Animal Research Ethics Committee (approval no. NWU-02003-20-A5) and of

the Federation of Animal Science Societies (FASS, 2010) for the care and use of agricultural animals in research as well as teaching.

4.3 Statistical analysis

One-way analyse of variance was used to analyse the data attained from the experiment using the GLM of SAS (2010) in a completely randomized design. The following model was employed:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where; Y_{ij} - The response variable, μ - The overall mean, D_i - Treatment effect, E_{ij} - Random error

For a statistical test, significance was declared at $P < 0.05$. The least-squares means were compared using the probability of difference (pdiff) option. Linear regression analysis was used to establish associations between chemical characteristics, relative palatability index, as well as intake. The following regression model was used:

$$Y = aX + b$$

Where; Y - dependent variable, a – constant, X - independent variable, b – intercept

4.4 Results

4.4.1 Chemical composition

Results of the chemical composition of experimental feeds are similar to those presented in Section 3.4.

4.4.2 Palatability indices

Feed intake and relative palatability indices of feeds by goats are presented in Table 4.1. Feed intake and relative palatability of feeds differed significantly ($P < 0.05$) among the goats. Lablab was consumed more ($P < 0.05$) than any other feed. However, goats consumed a similar ($P > 0.05$) quantities of maize and Dr Saunders. Goats consumed Betswit the least ($P < 0.05$). Lablab had a high palatability index ($P < 0.05$) when compared to other feeds. Maize straw had the similar ($P > 0.05$) palatability indices to Dr Saunders and Betswit leaves.

Table 4.1: Feed intake (Ti) and relative palatability index (Ri) by goats

Species	Intake (kg/d)	Palatability indices
Maize	0.27 ^b	1.00 ^{bc}
Dr Saunders	0.27 ^b	1.07 ^b
Lablab	0.36 ^a	1.42 ^a
Betswit	0.19 ^c	0.79 ^c
S.E	0.02	0.08
P-value	<0.0001	<0.0001

^{a, b, c, d} Column means with same superscripts do not differ ($P > 0.05$)

Regression equations predicting palatability and intake from CP, NDF, ADF, ADL, EE, CT, Phenols and palatability index of maize and legumes (Dr Saunders, Lablab, and Betswit) fed to goats are presented in Table 4.2. The palatability index was positively and significantly ($P < 0.05$) predicted from the intake of goats ($r^2 = 0.977$). Except for lignin ($r^2 = 0.867$) and phenols ($r^2 = 0.991$, $r^2 = 0.995$) there were negative associations between the chemical composition of diets and their palatability indices and intakes by goats.

Table 4.2: Regression equations predicting palatability and intake from CP, NDF, ADF, ADL, EE, CT, Phenols and palatability index of maize and legumes (Dr Saunders, Lablab, and Betswit) fed to goats

Factor	Y-variable	Formulae	R ²	Probability
CP	Intake	$0.229x + 0.00001$	0.067	0.740
NDF	Intake	$0.307x + - 8.333$	0.026	0.840
ADF	Intake	$0.539x + - 0.001$	0.194	0.559
ADL	Intake	$0.704x + - 0.003$	0.867	0.069
EE	Intake	$0.439x + -0.007$	0.792	0.110
CT	Intake	$0.305x + - 0.010$	0.164	0.595
Phenols	Intake	$0.130x + 0.640$	0.991	0.005
Intake	Palatability	$0.001x + - 0.252$	0.977	0.012
CP	Palatability	$0.829x + 0.001$	0.149	0.619
NDF	Palatability	$1.328x + - 0.001$	0.086	0.707
ADF	Palatability	$2.404x + - 0.004$	0.313	0.441
ADL	Palatability	$2.678x + - 0.011$	0.779	0.117
EE	Palatability	$1.677x + -0.027$	0.673	0.180
CT	Palatability	$1.156x + -0.030$	0.690	0.737
Phenols	Palatability	$0.515x + 2.514$	0.995	0.003

4.4.3 *In vitro* dry matter degradability

Results on the IVDMD of legumes and maize straws are presented in Table 4.3. Significant ($P<0.05$) variations were observed in IVDMD among the forages throughout different incubation times. Dr Saunders (300.9 g/kg DM) had higher ($P<0.05$) IVDMD6 than maize, Betswit, as well as lablab. Dr Saunders and lablab had the higher ($P<0.05$) IVDMD12 than maize and Betswit. Lablab and Dr Saunders had similar ($P>0.05$) (389.1, and 387.4 g/kg DM respectively) IVDMD24, while maize had the lowest ($P<0.05$) (290.0 g/kg DM) IVDMD24. Lablab (564.5 g/kg DM) and Dr Saunders (542.9 g/kg DM) IVDMD36 were similar ($P>0.05$) and higher ($P<0.05$) than other forages. Lablab and Dr Saunders had similar ($P>0.05$) (610.8 and 607.6 g/kg DM respectively) IVDMD48, which was higher when compared to Betswit and maize. Lablab had the highest ($P<0.05$) IVDMD (716.5 g/kg DM) at 72h while maize (496.3 g/kg) had the lowest IVDMD72 when compared to all other forages.

Table 4.3: *In vitro* dry matter degradability of legume leaves and maize straws (g/kg DM) (0, 6, 12, 24, 36, 48 and 72h)

Species	IVDMD0	IVDMD6	IVDMD12	IVDMD24	IVDMD36	IVDMD48	IVDMD72
Maize	173.8 ^c	206.6 ^c	229.5 ^c	290.0 ^c	360.3 ^c	431.9 ^c	496.3 ^d
Dr Saunders	240.2 ^a	300.9 ^a	385.7 ^a	387.4 ^a	542.9 ^a	607.6 ^a	687.7 ^b
Lablab	258.7 ^a	252.9 ^b	369.3 ^a	389.1 ^a	564.5 ^a	610.8 ^a	716.5 ^a
Betswit	205.3 ^b	245.5 ^b	322.2 ^b	357.8 ^b	491.5 ^b	575.0 ^b	662.5 ^c
S.E	7.85	7.73	7.01	6.59	8.63	7.01	7.27
P value	0.0003	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c,d} means with different superscripts in the same column are different (P<0.05), S.E: standard error, IVDMD0, 6, 12, 24, 36, 48, 72. *In vitro* dry matter degradability at 0, 6, 12, 24, 36, 48, and 72 hr

4.5 Discussion

4.5.1 Palatability indices

The palatability of legume forages is an important factor in ruminants. High palatability and intake of lablab and Dr Saunders by goats suggest that these legumes have a potential for utilization in livestock (sheep and goats) feeds (Ngwa et al., 2003; Osuga et al., 2008; Deng et al., 2017). However, palatability and intake of Betswit were the lowest; this was attributable to its high concentration of condensed tannins (CT). Similar to the current findings, Deng et al. (2017) stressed that lower intake and palatability index of legumes such as *Tamarindus indica* was due to high CT concentration when fed to goats. Low CT concentrations (20-40 g/kg) are favourable since they decrease ruminal protein degradation and increase absorption of essential amino acids (Mokoboki et al., 2011), leading to great livestock performance (Obour et al., 2015). High concentrations of CT (≥ 50 g/kg) in the forage adversely influence the preference, intake and palatability of forages, when offered to ruminants (Peng et al., 2016; Wang et al., 2018) which might be due to astringency and bitterness (Rojas-Hernandez et al., 2016; Brown et al., 2018; Sebolai, 2018). The astringent sensation that goats most possibly feel when eating Betswit may reduce its palatability (Gwanzura et al., 2011; Obour et al., 2015). The low palatability and intake of Betswit is perhaps a reaction by goats to the adverse influence of CT concentration on Betswit digestibility (Obour et al., 2015). In the present study, Betswit also had the highest ether extract (EE) content than other legumes. Obour et al. (2015) and Deng et al. (2017) stressed that *Broussonetia papyrifera* and *T. indica* were the least preferred species due to the high EE content in these species. Therefore, the high EE content of Betswit was attributable to its low palatability and intake. Forages with high EE content limit feed intake and interrupt the functioning of the rumen (Martinez Marin et al., 2013). Rumen microbes do not ferment fat and fat does not

contribute towards the growth of rumen microbes, thus reducing protein synthesis (Goulas *et al.*, 2001).

Except for ADL and total phenols (TP), all chemical parameters had poor association with both intake and palatability indices of diets by goats. The observed poor associations between chemical composition and palatability index and intake were also reported by Ravhuhali (2010) and Gwanzura *et al.* (2011). Dr Saunders with high CP content gave medium palatability and intake values in goats. Also, Rogosic *et al.* (2006) and Alonso-Diaz *et al.* (2008) reported that intake was not associated with CP content. Poor associations between the palatability index of goats and fibre content of leaves were also reported by Ikhimioya (2008). Total phenolics had a positive association with both intake and palatability values in the current study. These results are in contrast with the results reported by Alonso-Diaz *et al.* (2008), who reported that total phenolics were negatively correlated with palatability index and intake values. These differences might be caused by the different types of forage species used in the experiments. Lignin was negatively correlated with the palatability index, this was in agreement with the studies of Sanon *et al.* (2008) and Sultan *et al.* (2008). In the current study, intake had a strong relationship with palatability. These results are in agreement with the results reported by Ikhimioya (2008), Sultan *et al.* (2009), Gwanzura *et al.* (2011) and Brown *et al.* (2016).

4.5.2 *In vitro* dry matter degradability

In vitro dry matter degradability is a method used to estimate the dissipation of feeds in filter bags within the rumen (Cone *et al.* 1999). According to Ikhimioya *et al.* (2005), this technique is of importance as it gives information about the digestibility as well as the quality of rumen substrate to ruminant nutritionists. Castro *et al.* (2003) stated that *in vitro* procedures for assessment of feeds are a significant instrument as they permit speedy evaluation of nutritional value as well as possible

harmful action of whichever anti-nutritional compound that exists in the feed. In the current study, the degradation of substrates of all the forages used showed an increase as the incubation period was increasing and was in agreement with other studies. Previous researches by Muya *et al.* (2020) as well as Dinkale *et al.* (2021) also confirmed that there is an increase in DM disappearance of the forage as the incubation period increases. The results in the current study showed that lablab and Dr Saunders had a higher IVDMD than other forages throughout the incubation time. In the current study, lablab had higher IVDMD than Dr Saunders at 24, 36, 48 and 72 hours of the incubation period. In most cases, the 48th hr incubation period is a perfect measurement of *in vitro* DMD because many forages remain in the digestive system of livestock ruminants for that long (Dinkale *et al.*, 2021). The highest IVDMD of lablab could be attributable to its lower ADF as well as ADL (Rojas-Hernandez *et al.*, 2016; Muya *et al.*, 2020; Ravhuhali *et al.*, 2020).

According to Muya *et al.* (2020), a high ADF concentration in the forage reduces rumen microorganisms' ability to digest nutrients, thereby restricting microorganisms' "attack". Lignin is a multifaceted organic compound that reduces the nutritive value of forages for livestock ruminants by inhibiting the capacity of rumen microorganisms to break down and colonize plant cells (Ravhuhali *et al.*, 2020). Among all the legumes, Betswit was the least degradable legume. This was attributable to its high ether extracts as well as tannins. Forages high in EE content generally have low degradability. Muya *et al.* (2020) reported that forages with high EE content physically protect substrate, thus restricting rumen microbes' "attack". Tannins e.g. CT cause proteins to bind into complexes, which negatively affects protein degradation in the rumen (Addisu, 2016). In the present study, the low degradability of Betswit suggests a possible tannin-induced depression that were much higher for the same species in the previous chapter. Tannins may have bactericidal effects on rumen microbes thus reducing forage fermentation (Simbarashe *et al.*, 2015). Tannins

(CT), the defaunating effect can decrease the microbial population, thereby interfering with the normal degradability of forages (Wanapat *et al.*, 2012; Olivares-Perez *et al.*, 2013). Maize straw had the lowest IVDMD than all the plant types throughout incubation, this was in agreement with by Azim *et al.* (2000). The author stressed that non-legume forages such as sorghum, millet, and maize have lower degradability values when compared to legumes. The low degradability of low-quality roughages such as maize could be associated with its low CP content when compared to high-protein forages such as legumes (Oh *et al.*, 2008; Mlambo *et al.*, 2009). When there is high CP diet in the rumen it is normally exposed to microorganisms which can attack and produce amino acids, ammonia and peptides, and these products are then used for the synthesis of microbial protein.

4.6 Conclusion

Among the legumes, lablab had higher degradability and palatability. Except for phenols, all chemical composition parameters poorly predicted intake and palatability, which implies that chemical composition is a poor predictor of palatability and intake. The legumes showed high IVDMD compared to maize straw. However, though having high phenols, based on the higher degradability and relative palatability, lablab can be recommended to supplement low-quality roughages such as grass and straws due to high CP content and lower lignin.

4.7 References

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Chapter 5: Effect of inclusion of legume leaves on nutritive value, *in vitro* dry matter degradability, fermentation characteristics and aerobic stability of maize straws silage

Abstract

The objective of this study was to assess the effect of the inclusion of legumes on nutritive value, *in vitro* dry matter degradability, fermentation characteristics and aerobic stability of maize straw silage. For silage, the proportion of legume to maize was 20:80%. Treatments were as follows, maize-lablab (ML) (20:80%), maize-indigenous cowpea (MI) (20:80%), maize-betswit (MB) (20:80%) and maize-Dr Saunders (MD) (20:80%), each with molasses (2%) to enhance fermentation. The controls were maize, without (negative control) or similarly treated with molasses (positive control). Molasses was diluted with distilled water at 1:2. The mixtures were then ensiled for 45 days following the ensiling period, then were opened and analysed for chemical composition analysis and fermentation characteristics. Other samples were put through a 7-day aerobic stability test. All data were subjected to a one-way analysis of variance (SAS, 2010) in a completely randomized design. For the nutritive value of the silage, maize-molasses had the highest DM and hemicellulose content than all other silages. Maize-Dr Saunders had the highest ($P<0.05$) ash, and EE content when compared to all other silages. Maize-lablab silage (116.0 g/kg DM) had the highest CP content when compared to other silages. Maize had the highest ($P<0.05$) NDF (619.2 g/kg DM) and ADL (140.0 g/kg DM) content when equated with all other silages. The ML and MD had the highest ($P<0.05$) ADF content (394.7 and 393.6 g/kg DM respectively) than other silages. The ML had the highest ($P<0.05$) content of cellulose when compared to other silages. The highest ($P<0.05$) IVDMD was recorded for ML silage from 24 hrs up to 72 hrs. The highest ($P<0.05$) pH was recorded for MB. All silages had similar ($P>0.05$) concentration of LA.

The MM had the highest ($P<0.05$) content of WSC and ML had the least ($P<0.05$) content of WSC. The ML had the highest ($P<0.05$) LAB counts than all other silages. The yeast and mould counts of all silages were similar ($P>0.05$). A higher CO_2 was recorded for MB than all silages. The addition of legumes, as well as molasses in maize straw, resulted in improved silage quality and fermentation characteristics.

Keywords: legumes, fermentation characteristics, maize, nutritive value, rumen fermentation, silage

5.1 Introduction

Silage is the product after a sequence of biochemical processes in which a forage with moisture content is cut and fermented to form a stable feed that will not degrade further in anaerobic storage (Amuda & Tanko, 2019). Maize (*Zea mays*) silage is one of the most used feeds across the world, due to its high-energy content. However, it contains low CP content, less than 7 g/kg DM (Contreras-Govea *et al.*, 2009). To meet the nutrient requirements for ruminants, the CP of maize straw silage must be increased to required levels (Zhu *et al.*, 2011). Goyal & Tiwana (2016) stated that legume leaves are a good source of CP and minerals. However, legumes cannot be ensiled alone due to their high buffering capacity as well as their low WSC (Ozturk *et al.*, 2006; Artabandhu, 2018) with risks of producing butyric acid (Heinritz *et al.*, 2012). Mixing maize and legumes for silage production is a potential strategy for increasing the CP of maize silage (Zhu *et al.*, 2011; Gulumser *et al.*, 2021). Legume leaves and maize straws could be ensiled to provide adequate fodder for ruminant production throughout the dry periods.

According to Phiri *et al.* (2007) and Campbell *et al.* (2020), ensiling causes reductions in fibre fractions of silages, due to the activity of enzymes throughout fermentation. Studies of Ozturk *et*

al. (2006) and Pursiainen & Tuori (2008) have equated a variety of legume-cereal mixtures in relation to fermentation characteristics, and have revealed that the proportion of legume to cereal has manifest impacts on fermentation.

The *in situ* nylon bag method (Ørskov & McDonald, 1979) is the most commonly used method for nutrient degradation kinetics (Nkosi *et al.*, 2019). As a result, ruminant feed degradability is crucial for evaluating feed ingredient utilization (Lei *et al.*, 2018). The data generated by *in vitro* ruminal fermentation procedures can be used to select forages or feeds that digest and degrade quicker, allowing ruminant production to be maximized. There is a need to continue evaluating the forage conservation methods and their impact on addressing feed shortages during the dry season. Therefore, the objective of this study was to investigate the influence of the inclusion of legumes in maize straw on the nutritive value, *in vitro* dry matter degradability, and ensiling characteristics of maize straw silage.

5.2 Material and methods

5.2.1 Study site

Same as described in section 3.2.1

5.2.2 Silage preparation

Freshly harvested and chopped whole maize straws and legume leaves were used as raw materials for the study. After harvesting, each legume and the maize were sliced into 3 cm pieces by a motorized chopper and each legume mixed with the maize at 20:80% proportion (Ngongoni *et al.*, 2008). Treatments were as follows, maize-lablab (ML) (20:80%), maize-indigenous cowpea (MI) (20:80%), maize-betswit (MB) (20:80%) and maize-Dr Saunders (MD) (20:80%), each with molasses (2%) to enhance fermentation. The controls were maize, without (negative control) or

similarly treated with molasses (positive control). Molasses was diluted with distilled water at 1:2. A kilogram of each treatment was thoroughly mixed within plastic sheets prior to packing in polythene bags. Each treatment was replicated 5 times. Compaction as well as the elimination of air from the bags was accomplished by hand squeezing, to eliminate as much air as possible, and then to tie using twine to ensure air-tightness. The silages were opened subsequently 45 days later. Samples from the silages were taken to determine silage chemical composition (DM, ash, CP, EE, NDF, ADF and ADL; hemicellulose and cellulose were calculated using the method described in Chapter 3) after ensiling and the fermentation characteristics (pH, lactic acid, water soluble carbohydrates, lactic acid bacteria, yeast and mould, and carbon dioxide).

5.2.3 Chemical analysis, *in vitro* dry matter degradability, fermentation characteristics and aerobic stability determination

Chemical analysis of pre ensiled mixtures and that of silages was analysed as indicated in section 3.2.4. *In vitro* dry matter degradability was determined as detailed in section 4.2.2.

The pH of the silages was determined using an Orion digital pH meter 611 on the silage aqueous extract. The LA was determined utilizing the colourimetric technique of Barker & Summerson (1941) as improved by Pryce (1969). The WSCs were determined utilizing the phenol-sulphuric acid technique of Dubois *et al.* (1956). Yeasts plus mould counting were done on a potato dextrose agar spread-plate acidified with LA (85%). The plates were incubated at 28 °C, the yeasts were counted at 48 hours, and the counting mould was counted at 96 hours.

Silage (500 g) samples were loosely packed in an open plastic jar to determine aerobic stability, which was shielded with 2 layers of cheesecloth and stowed at room temperature. The production

of CO₂ (Ashbell *et al.*, 1991), was determined after the 7-day aerobic exposure as described by the IDF (1990) technique.

5.3 Statistical analysis

The general linear model (GLM) procedure of SAS (2010) was carried out to work out data on the effect of inclusion of legumes in maize silage on nutritive value, *in vitro* dry matter degradability, fermentation characteristics and aerobic stability. The following GLM procedure was be used:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where; Y_{ij} - The response variable, μ - The overall mean, D_i - Treatment effect, E_{ij} - Random error

For a statistical test, significance was declared at $P < 0.05$. The least squares means were compared using the probability of difference (pdiff) option.

5.4 Results

The results of the chemical composition of maize–legume silage at pre-ensiling are presented in Table 5.1. The highest ($P<0.05$) DM value was observed for M. The MI and M had the high ($P<0.05$) ash contents (165.5 and 164.2 g/kg DM respectively). Maize-lablab had the highest ($P<0.05$) CP content when equated to other pre-ensiled mixtures. The highest ($P<0.05$) EE content was observed for ML.

Table 5.1: Chemical composition (g/kg DM) of maize–legume mixed silage at pre-ensiling (n=5)

	DM	Ash	CP	EE
M	915.2 ^a	164.2 ^a	70.6 ^c	19.7 ^c
MM	903.1 ^c	137.7 ^d	87.1 ^b	24.6 ^{bc}
MD	902.1 ^c	146.7 ^c	116.5 ^a	39.4 ^a
ML	896.9 ^d	155.1 ^b	119.5 ^a	37.7 ^a
MI	893.1 ^d	165.5 ^a	103.0 ^b	25.0 ^{bc}
MB	910.5 ^b	147.6 ^c	105.4 ^b	27.0 ^b
SE	1.32	1.82	1.44	1.95
P-value	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c,d,e} Means in the same column with different superscripts are different ($P<0.05$). DM: dry matter, EE: ether extract, CP: crude protein, SE: standard error, M: maize alone, MM: maize-molasses, MD: maize-Dr Saunders, ML: maize-lablab, MI: maize-indigenous cowpea, MB: maize-Betswit

The results of fibre proportions of maize-legume mixed silage at pre ensiling are presented in Table 5.2. Maize silage had the highest ($P<0.05$) NDF content. The MD had the highest ($P<0.05$) ADF content. The highest ($P<0.05$) ADL content was observed for MB (146.2 g/kg DM) while the lowest ($P<0.05$) was observed for MM (110.0 g/kg DM). Maize-Dr Saunders had the highest ($P<0.05$) content for cellulose (274.3 g/kg DM). The highest ($P<0.05$) hemicellulose was recorded for MM.

Table 5.2: Fibre proportions (g/kg DM) of maize–legume mixed silage at pre ensiling (n=5)

	NDF	ADF	ADL	Cel	Hemi
M	622.6 ^a	387.6 ^c	141.2 ^b	246.4 ^b	235.0 ^a
MM	615.0 ^a	362.9 ^d	110.0 ^b	252.9 ^b	252.1 ^a
MD	534.8 ^{cd}	416.3 ^a	141.9 ^a	274.3 ^a	118.6 ^c
ML	547.2 ^{bc}	408.9 ^a	136.1 ^a	272.8 ^a	138.3 ^c
MI	530.1 ^d	403.5 ^{ab}	134.3 ^a	269.2 ^a	126.6 ^c
MB	555.9 ^b	393.7 ^{bc}	146.2 ^a	247.5 ^b	162.3 ^b
SE	4.39	4.69	5.54	4.39	6.65
P-value	<0.0001	<0.0001	0.0076	0.0011	<0.0001

^{a,b,c,d,e} Means in the same column with different superscripts are different ($P<0.05$). NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, Cel: cellulose, Hemi: hemicellulose, SE: standard error, M: maize alone, MM: maize-molasses, MD: maize-Dr Saunders, ML: maize-lablab, MI: maize-indigenous cowpea, MB: maize-Betswit

The results of the chemical composition of the silages are presented in Table 5.3. There was a significant variation ($P<0.05$) in the chemical composition between silages. Maize-molasses (MM) (885.9 DM) had the highest ($P<0.05$) DM content than all other silages. Maize-Dr Saunders had the highest ($P<0.05$) ash and EE content when compared to all other silages. The ML (116.0 g/kg DM) had the highest ($P<0.05$) CP content and M (69 g/kg DM) had the lowest ($P<0.05$) CP content when compared to all other silages.

Table 5.3: Chemical composition of silages (g/kg DM) after 45 days of ensiling (n=5)

	DM	Ash	CP	EE
M	865.4 ^b	135.8 ^d	69.0 ^f	24.9 ^b
MM	885.9 ^a	147.1 ^c	84.1 ^e	22.7 ^{bc}
MD	845.6 ^c	160.9 ^a	112.8 ^b	35.2 ^a
ML	806.9 ^e	138.7 ^d	116.0 ^a	22.8 ^{bc}
MI	816.3 ^d	153.0 ^b	97.7 ^d	21.0 ^c
MB	887.9 ^a	145.4 ^c	102.8 ^c	24.3 ^{bc}
S.E	1.38	1.72	0.75	1.17
P-value	<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c,d,e} Means in same column with different superscripts are different ($P<0.05$). DM: dry matter, EE: ether extract, CP: crude protein, SE: standard error, M: control maize, MM: control maize-molasses, MD: maize-Dr Saunders, ML: maize-lablab, MI: maize-indigenous cowpea, MB: maize-betswit.

The results for fibre proportions of silages are presented in Table 5.4. Maize (619.2 g/kg DM) had the highest ($P<0.05$) NDF content, while MB (493.1 g/kg DM) had the least ($P<0.05$) content of NDF when equated with all other silages. The ML and MD had the highest ($P<0.05$) ADF content (394.7 and 393.6 g/kg DM, respectively) than other silages. Maize (140.0 g/kg DM) had the highest content of ADL compared to other silages. The ML had the highest ($P<0.05$) content of cellulose when compared with other silages. Maize-molasses had the highest ($P<0.05$) content of hemicellulose than all other silages.

Table 5.4: Fibre proportions of silages (g/kg DM) after 45 days of ensiling (n=5)

	NDF	ADF	ADL	Cel	Hemi
M	619.2 ^a	385.7 ^b	140.0 ^a	245.7 ^b	233.5 ^a
MM	591.6 ^b	343.8 ^d	101.7 ^c	242.1 ^b	247.8 ^a
MD	510.5 ^{cd}	393.6 ^a	126.8 ^b	266.8 ^a	116.9 ^b
ML	515.5 ^c	394.7 ^a	124.4 ^b	270.3 ^a	120.8 ^b
MI	510.1 ^{cd}	389.9 ^{ab}	128.9 ^{ab}	261.0 ^a	120.2 ^b
MB	493.1 ^d	365.2 ^c	118.6 ^b	246.5 ^b	128.0 ^b
S.E	5.50	2.02	3.78	4.46	5.98
P-value	<0.0001	<0.0001	0.0003	0.0023	<0.0001

^{a,b,c,d,e} Means in same column with different superscripts are different ($P<0.05$). NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, Cel: cellulose, Hemi: hemicellulose, SE: standard error, M: control maize, MM: control maize-molasses, MD: maize-Dr Saunders, ML: maize-lablab, MI: maize-indigenous cowpea, MB: maize-betswit.

The results for *in vitro* dry matter degradability are presented in Table 5.5. Maize-lablab had the highest ($P<0.05$) IVDMD value (343.6 g/kg DM) when compared to other silages at 24h. The ML (469.1 g/kg DM) had the highest ($P<0.05$) IVDMD36 value when compared to other silages. The ML (497.9 g/kg DM), MI (493.3 g/kg DM) and MM (488.5 g/kg DM) had the highest ($P<0.05$) IVDMD48 values. Maize-lablab, MM, and MI had the highest ($P<0.05$) IVDMD72 values (628.5, 618.5 and 614.2 g/kg DM, respectively).

Table 5.5: *In vitro* dry matter degradability of silages (g/kg DM) (0, 6, 12, 24, 36, 48 and 72h)

	DMD0	DMD6	DMD12	DMD24	DMD36	DMD48	DMD72
M	201.6 ^b	230.2 ^c	236.4 ^b	296.2 ^d	391.2 ^d	482.4 ^{ab}	544.9 ^d
MM	221.5 ^a	257.8 ^a	266.8 ^a	333.2 ^{ab}	434.8 ^b	488.5 ^a	618.5 ^a
MD	215.5 ^{ab}	227.8 ^{dc}	243.8 ^b	333.4 ^{ab}	411.8 ^{cd}	469.0 ^b	591.6 ^b
ML	209.3 ^{ab}	231.7 ^{bc}	251.6 ^{ab}	343.6 ^a	469.1 ^a	497.9 ^a	628.5 ^a
MI	202.3 ^b	218.5 ^d	237.5 ^b	312.9 ^c	422.7 ^{bc}	493.3 ^a	614.2 ^a
MB	219.7 ^a	241.2 ^b	255.2 ^{ab}	326.8 ^{bc}	433.1 ^{bc}	469.8 ^b	570.0 ^c
S.E	4.69	3.20	6.29	5.04	6.68	5.21	6.07
P-value	0.0387	<0.0001	0.0387	0.0003	<0.0001	0.0080	<0.0001

^{a,b,c,d,e} means with same superscripts in the same column are not different ($P>0.05$). S.E: standard error, IVDMD 0, 6, 12, 24, 36, 48, 72. *In vitro* dry matter degradability at 0, 6, 12, 24, 36, 48, and 72 hr, M: maize (control), MM: maize-molasses (control), MD: maize-Dr Saunders, ML: maize-lablab, MI: maize-indigenous cowpea, MB: maize-betswit.

The results for fermentation characteristics as well as aerobic stability are presented in Table 5.6.

The highest ($P<0.05$) pH was recorded for MB. All silages had similar ($P>0.05$) concentration of

LA. The MM had the highest ($P<0.05$) content of WSC and ML had the least ($P<0.05$) WSC content. The ML had the highest ($P<0.05$) LAB counts than all other silages. The yeast and mould counts of all silages were similar ($P>0.05$). A higher CO_2 was recorded for MB than all silages.

Table 5.6: Effect of legume residue on fermentation characteristics and aerobic stability (g/kg DM, unless stated otherwise) of maize straw after 45 days of ensiling (n=5)

	Fermentation characteristics					Aerobic stability
	pH	LA	WSC	LAB (cfu/g)	Y & M (cfu/g)	CO_2
M	3.66 ^d	43.3 ^a	12.0 ^{bc}	6289 ^{bc}	<10	6.17 ^c
MM	4.43 ^c	63.7 ^a	17.7 ^a	26911 ^{ab}	<10	3.41 ^c
MD	4.51 ^{bc}	48.0 ^a	12.6 ^{abc}	30156 ^{ab}	<10	13.18 ^b
ML	4.58 ^{abc}	44.0 ^a	9.3 ^c	35167 ^a	<10	9.04 ^{bc}
MI	4.68 ^{ab}	45.3 ^a	11.0 ^{bc}	17667 ^{abc}	<10	5.43 ^c
MB	4.75 ^a	65.3 ^a	16.0 ^{ab}	660 ^c	<10	20.49 ^a
SE	0.06	7.70	1.70	7439.25		1.78
P-value	<0.0001	0.2037	0.0379	0.0375		0.0002

^{a,b,c,d,e} Means in same column with different superscripts are different ($P<0.05$). pH: potential of hydrogen, LA: lactic acid, WSC: water soluble carbohydrates, LAB: lactic acid bacteria, Y & M: yeast and moulds, CO_2 : carbon dioxide, M: control maize, MM: control maize-molasses, MD: maize-Dr Saunders, ML: maize-lablab, MI: maize-indigenous cowpea, MB: maize-betswit, SE: standard error

5.5 Discussion

5.5.1 Nutritive value of the silage

Silage DM is the key predictor of effluent losses and effluent is avoided in most circumstances when DM content is more than 35% (Batista *et al.* 2019). Ensiling of maize straw resulted in reduced DM contents of silages. The DM contents of legume-maize silages ranged from 806.9-887.9%. This could be ascribed to a technique used to determine the DM content of the silages (Mokoboki *et al.*, 2016). According to Kunkle *et al.* (2006), another reason could be the production of water throughout fermentation.

According to Ahmed *et al.* (2013), the ash content found in feeds plays a vital role in stimulating balanced animal growth. The ash content of silages in the current study ranged between 135.8 and 160.9 g/kg DM. These values were found to be higher than the range (5.90-7.00% DM) reported by Edson *et al.* (2018) on maize and maize-legume silages. The higher ash content in the present study could be ascribed to high levels of minerals in the legumes used which were reported by the studies of Soetan & Fafunso (2010), Baloyi *et al.* (2013) and Yusuf *et al.* (2017). This could be an added benefit when supplemented to ruminants fed on roughages of poor-quality that are lacking minerals (Gusha *et al.*, 2015). The higher ash content in these silages suggests that there may be no necessity to supplement with commercial minerals if these silages are used. Conversely, Mugweni *et al.* (2006) stated that there would be a necessity to analyze for minerals required by ruminants to determine the adequacy of these silages.

The CP content (69 g/kgDM) obtained in this study for maize silage (without molasses) was similar to 69 g/kg DM reported by Phiri *et al.* (2007) and Contreras-Govea *et al.* (2009) and was lower than 9.99% DM obtained by Young *et al.* (2012). The differences in maize varieties, the phase at which the crop was harvested, as well as the duration of the ensiling process could be ascribed to

the differences. The CP content of a feed is one of the most critical factors in improving ruminant performance, and low concentrations can have a detrimental impact on animal performance. Moreover, silages with CP content of <70 g/kg DM do not promote optimal rumen fermentation and may lead to reduced feed intake (Malebana *et al.*, 2019). In the current study, the CP content of MM, MD, MB, MI and ML was greater than this threshold, which is considered enough for optimal ruminal microorganisms' activity. This is a sign that maize ensiled alone could not be considered as a sole feed. The inclusion of legumes in maize increased the CP content of the silages. This did not come as a surprise since previous studies have shown that the addition of legumes in maize increases CP content. These results were similar to the results of Contreras-Govea *et al.* (2009) and Stoltz *et al.* (2013) where the inclusion of legumes in maize increased CP in the silages. The CP content of ML (116.0 g/kg DM) was similar to the 113 g/kg DM, which is considered as a proposed minimum requirement for growth in ruminants (ARC, 1984).

The EE content of silages except for MD from this study was similar to the values obtained by Htet *et al.* (2016). The EE content of MD (35.2 g/kg DM) was comparable to the EE content (33.3 and 30.1 g/kg DM) obtained by Erdal *et al.* (2016) and Tharangani *et al.* (2021), respectively. The EE contents of all silages in the present study were also similar to the values obtained by Qu *et al.* (2013) and Edson *et al.* (2018) from maize-legume silages. The EE content in these silages shows that the silages have high-energy content and can be used as a source of energy in ruminant nutrition (Nkosi *et al.*, 2016a). Nevertheless, silages high in EE content can also reduce ruminant feed intake and disrupt the normal functioning of the rumen (Mciteka, 2008).

The inclusion of legumes in maize resulted in decreased fibre concentration in the silages. The current observations agreed with those by Phiri *et al.* (2007), who reported that the addition of legumes in maize reduced the fibre concentrations after ensiling. This could be due to

hemicellulose hydrolysis into monosaccharides, which offer extra carbohydrates (sugar) for lactic acid (LA) generation throughout fermentation. The reduced fibre concentrations in silage might lead to increased DM intake as well as digestibility since fibre concentrations have a negative association with DM intake as well as digestibility (Mciteka, 2008; Erdal *et al.*, 2016; Abdullah *et al.*, 2017). The NDF content of maize-legume silage ranged between 493.1-515.5 g /kg DM which is lower than the fresh (recorded in the previous chapter) and the ensiled maize straws. According to Msiza *et al.* (2021) this range indicates better feed intake. The NDF content (493.1 g/kg DM) of MB was similar to the NDF content (491 g/kg DM) obtained by Phiri *et al.* (2007) in *Leucaena leucocephala*-maize silage. The NDF content (515.5 g/kg DM) of ML was similar to the NDF content (531 g/kg DM) obtained by Balezentiene & Mikulioniene (2006) in galega-maize silage. Erdal *et al.* (2016) stated that the ADF content of silages is usually between 25-50%. The current results for ADF content in the silages ranged from 343.8 to 394.7 g/kg DM and it falls between the reported range. Since low concentrations of fibre constituents' aid for better digestion, it appears that the addition of legumes in maize outperformed the controls in terms of ADF and NDF.

The ADL contents of the silages were lower than the values obtained by Amole *et al.* (2013) and Contreras-Govea *et al.* (2013) from maize-lablab and maize-cowpea silage. This variation might be caused by the stage at which the forages were harvested, as well as the duration of fermentation. The lignin concentration in the substrates or silage might be shadowed by high CP content from legumes and this might stimulate the increase in microbes to breakdown lignin in the diet. Cattle have greater amounts of microbes in their digestive tract than sheep and goats, allowing them to properly break down the extremely lignified silages, which are extremely resilient to chemical and enzymatic degradation (Msiza *et al.*, 2021; Ravhuhali *et al.*, 2021). Therefore, feed intake will be increased in all silages due to their low lignin content.

5.5.2 *In vitro* dry matter degradability

The results in the current study showed that the silages had lower IVDMD values when compared with the legumes in Chapter 4. The lower IVDMD for silages could be ascribed to a proportion of maize fibre fractions found on the silages. Melaku *et al.* (2010) stated that the fibre fractions influence degradability negatively. The addition of legumes in maize and maize with molasses in the current study resulted in the higher degradability of the silage than the control silage (maize alone). This can be explained by a slight increase in CP content, as this nutrient is highly digestible (Stoltz *et al.*, 2013). The ML silage had high IVDMD values at 24 to 72 h, the CP content of this silage might have contributed to the high IVDMD of this silage. Lablab provided a source of N to microorganisms, which leads to high fibre degradation and thus high IVDMD (Mbukwane, 2017). Feeds with high CP content promote microbial proliferation, which affects the degree of fermentation (Mpanza *et al.*, 2020).

The observed increase in degradability of maize silage with molasses (positive control) when compared to maize only (negative control) was consistent with the observations from other studies (Sahoo & Walli, 2008; Besharati *et al.*, 2017) stating that adding molasses increases IVDMD. The use of molasses at favours rumen microbial population, thus enhancing silage degradation. Furthermore, molasses addition likely improved degradability by providing a favourable environment for rumen microbes through high rumen fermentation of molasses sugars (Sahoo & Walli, 2008). A higher IVDMD for maize silage than the fresh maize forage from Chapter 4 was observed. In agreement with Peyrat *et al.* (2014), the DM was degraded more speedily in maize silage than un-ensiled maize. The higher proportion of the quicker degradable fraction in ensiled maize explains this (Gerlach *et al.*, 2018).

5.5.3 Fermentation characteristics and aerobic stability

The silage pH is a major indicator of good silage when assessing silage quality, generally, the lower the pH, the greater the conservation and the more stable the silage (Jusoh *et al.*, 2016). The silage pH values ranged from 3.66 to 4.75 and these pH low values are a sign of well-preserved silage. Gusha *et al.* (2013) and Matlabe (2020) stressed that pH values lower than 4.8 could be considered appropriate for good quality silage production. The addition of legumes resulted in pH increment; this was in agreement with the findings by Carpici (2016). The increment of the pH values was ascribed to low DM and high buffering capacity of the legumes, primarily due to cation existence (Phiri *et al.*, 2007; Wang *et al.*, 2017; Amuda & Tanko, 2019; da Silva Brito *et al.*, 2020). Furthermore, when cations encounter organic acids produced during fermentation, they neutralize them, preventing a pH drop (Pirhofer-Walzl *et al.*, 2011; da Silva Brito *et al.*, 2020).

According to Grant & Ferraretto (2018), LA is the key fermentation end product in silage. Even though there was no significant difference among the treatments on LA concentration, the addition of legumes in maize in the present study slightly increased LA content in the silages compared with the control (negative). This was consistent with studies by Htet *et al.* (2016) and Wang *et al.*, (2017). The inclusion of legumes showed that legumes could stimulate LA, which might be linked to the associated influence of bacterial community from diverse forages (Wang *et al.*, 2017). Another reason for an increase in LA content of the silage could be ascribed to the high buffering capacity of legumes. Generally, legumes have a high buffering capacity, which results in high concentration of LA during fermentation required to decrease the pH value for good silage preservation (Tao *et al.*, 2020), and the pH from this study was within the recommended for good quality silage.

Silage aerobic stability is a critical aspect in ensuring that the ruminants receive well-preserved nutrients with the least amounts of toxins and mould (Wilkinson & Davies, 2013). Increased CO₂ production in silage shows yeast and mould activity, which raises the temperature and decreases silage quality (Nkosi *et al.*, 2016b). Yeast and mould utilize residual LA and WSC for their metabolism (Mutavhatsindi *et al.*, 2018). With the addition of molasses and legumes, the aerobic stability of silages deteriorated. Based on the current findings, ML and MI silages were stable, compared with MB and MD; according to Wilkinson & Davies (2013), silages that produce CO₂ less than 10 g/kg DM over 5 days are declared stable.

5.6 Conclusion

In the present study, maize-lablab silage performed better in terms of CP, IVDMD (24-72 hr). All silages had high ash content, which could be of benefit when fed to ruminants given roughages lacking minerals, and this suggests that there might be opportunity to reduce the commercial mineral supplements when these silages are used. Overall, ensiling improved maize degradability in all withdrawal periods. The silages had low pH values, which is a good indication of well-preserved silage. The inclusion of legumes showed that legume leaves could stimulate LA production, which could influence the bacterial community from diverse forages and can be ascribed to the high buffering capacity of legumes. Further studies are required to assess the effect of the inclusion of legumes on maize straws silage on livestock performance.

5.7 References

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Chapter 6: General discussion, conclusions and recommendations

Ruminant livestock can convert low quality forage into quality food products. Proximate analysis can provide a good indication of forage nutritive value. Chapter 3 evaluated the nutritive and predicted chemical estimates from chemical composition parameters of legume leaves and maize straws. Based on the findings, all the legumes had CP content greater than 150 g/kg DM, this was in agreement with other studies (Washaya *et al.*, 2018; Wangila *et al.*, 2021). The outcomes showed that lablab, indigenous cowpea, and Dr Saunders had more chemical constituents such as CP, CT and predicted DE and ME values. The evaluation suggested these legumes can be used to supplement low-quality roughages such as maize straws and grasses (Kiraz, 2011; Shetty *et al.*, 2017). The NRC (2007) also highlighted that the CP content of these legumes is more than enough of the minimum CP requirements for growing beef cattle, fattening and early-weaned lambs.

Chapter 4 evaluated the effect of legume leaves and maize straws on *in vitro* dry matter degradability, and palatability indices. The results showed that lablab was highly degradable. This was ascribed to its low ADF, ADL and CT. The CP content of a forage is regarded as one of the parameters that affect its degradability. However, in chapter 3, this was not the case for the degradability of legumes. Factors such as buffer solution preparation, feed processing or equipment handling could be attributable to the differences (Mahwasane, 2018). Tannins also influence degradability. They reduce degradability resulting in a slower rate of digesta passage (Tripathi *et al.*, 2006). Muya *et al.* (2020) also stressed that forages with high ADF concentration decrease ruminal microflora ability to digest nutrients, since it acts as a mechanical barrier

hindering ruminal microbes' action. According to Ravhuhali *et al.* (2020), lignin inhibits the capacity of rumen microorganisms to break down and colonize forage cells.

The results revealed that lablab was consumed more than other forages. The higher palatability and intake values of lablab were attributable to its low lignin. The condensed tannins (CT) found on Betswit might have depressed feed intake and palatability of this species. Reports from literature emphasised that when $CT \geq 5\%$, they have adverse effects on palatability, due to astringency and bitterness (Brown *et al.*, 2018). A negative relationship was found between palatability and lignin, this was in agreement with other studies (Sanon *et al.*, 2008; Sultan *et al.*, 2008).

Chapter 5 assessed the influence of the inclusion of legume leaves in maize straw on nutritive value, *in vitro* dry matter degradability and ensiling characteristics of maize straw silage. An improvement in CP content of silages was observed, as legume leaves were included in maize straws and this was in agreement with other studies. The increment in CP content of the silages could also be related to added molasses in silage due to its high CP content (Matlabe, 2020). The CP content of ML surpassed 113 g/kg DM regarded as a minimum requirement for growth in ruminants (ARC, 1984) and lambs (fattening) (NRC, 2007).

The outcomes of the present study revealed that fibre constituents are reduced during fermentation especially with the addition of legume leaves. Phiri *et al.* (2007) stressed that this might be due to cell wall hydrolyses caused by LA produced by bacterial enzymes throughout fermentation. The reduced fibre constituents might lead to increased feed intake and digestibility as these two factors are adversely correlated with fibre fractions (Matlabe, 2020).

The outcomes displayed that ensiling maize straws alone and with the addition of molasses and legumes can improve silage quality and increase ruminal degradability thus improving animal production throughout the dry season. This was ascribed to high CP content when legume leaves and molasses were added into maize straws, they provided a nitrogen source to microbial microflora that resulted in a greater fibre degradation and therefore high IVDMD (Mbukwane, 2017). Ensiled maize straw performed better than un-ensiled maize straw in terms of IVDMD. Gerlach *et al.* (2018) stated that this can be explained by higher proportions of faster degradable proportions in ensiled maize.

From fermentation characteristics, the outcomes showed that the addition of legume leaves on maize straws increased the pH as well as LA content of the silages. This could be attributed to the high buffering capacity of legumes (Tao *et al.*, 2020). All the silages in the current study had pH < 4.8, this value was considered suitable for good production of quality silage (Matlabe, 2020). The results revealed that the addition of legume leaves to maize straws increased CO₂ production to detrimental levels in MB and MD. This was ascribed to yeast and mould activity, which elevate temperature and decrease silage quality (Nkosi *et al.*, 2016).

Among all the legumes, lablab performed better in most parameters and can be highly recommended to supplement low-quality roughages such as grass and straws due to high CP content and lower lignin, more especially during the dry season. Further studies are required to evaluate the effect of inclusion levels of legumes on maize straw silage on livestock performance. More work is necessary to assess the effect of anti-nutritional factors of legumes on the performance of small ruminants.

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