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Proximate Analysis, *in vitro* Dry Matter Degradability and Palatability Index of Legume Residues and Maize Straws for Ruminants

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ABSTRACT

Background: Legume residues such as leaves from cowpea and lablab plants can be accumulated in large quantities after fruits harvesting. These legumes can be incorporated in ruminants' diets due to their high feeding value. They are rich in crude protein, vitamins and minerals when compared to grasses that normally deteriorate during dry seasons. This paper is focusing on determining effect of legume residues and maize straws on *in vitro* dry matter degradability (IVDMD) and palatability indices in ruminants.

Methods: For palatability experiment, each of five male boer goats was provided with four feeding troughs carrying four different feeds (maize straws and 3 legumes = *Lablab purpureus* - lablab and 2 *Vigna unguiculata* - cowpeas - Dr Saunders and Betswit). The legume residues and maize straws were also analyzed for chemical composition and IVDMD. All data was subjected to a one-way analysis of variance.

Result: Dr Saunders (230.7 g/kg DM) had the highest (P<0.05) crude protein content when compared to all diets. Lablab (126.3 g/kg DM) had the lowest (P<0.05) acid detergent lignin. The highest (P<0.05) condensed tannins concentrations (50.1 g/kg DM) was recorded for Betswit. The highest (P<0.05) *in vitro* dry matter degradability was recorded for lablab at 24 up to 72 hrs. Lablab had a higher palatability index (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 on goats. Though 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.

Key words: Degradability, Goats, Intake, Legumes, Nutritive value, Palatability, Regression.

INTRODUCTION

Ruminant production in the tropics is restricted attributable to poor nutrition triggered by insufficient quantities and poor-quality roughages found in the rangelands, especially in winter. During the dry season, available pastures are of poor-quality with protein being the main restrictive nutrient (Buthane *et al.,* 2021). Commercial protein supplements tend to be expensive, as a result, there's a need to supplement the available pastures with cheap protein supplements like legumes. Moreover, conserving forages of high-quality found during the rainy season is another method that reduces the costs of purchasing protein concentrates (Ngongoni *et al.*, 2008, Dutta *et al.*, 2021). Apart from improving soil properties (Suwarto, 2021), legumes have great potential as it can be used as a fodder as well as a protein supplement for livestock *e.g.* goats, sheep as well as cattle (Nair *et al.,* 2021) . However, their intake is influenced by the acceptability, palatability, or preference by goats. Chemical compounds such as phenolics, alkaloids, tannins and aromatic compounds found in legume plants have been shown to affect feed's palatability, intake, degradability regardless of its nutritional content (Ravhuhali *et al.*, 2011, Obour *et al.*, 2015). Deng *et al.* (2017) also stated that the intake and relative palatability index of a feed could also be influenced by the protein and fibre content of a feed.

The palatability of legumes is a significant factor in the production of goats, especially if the legumes consumed, as it provides a key part of the daily nutrient intake. Mokoboki *et al.* (2011) stated palatability of a legume depends on its ability to stimulate an animal's organoleptic sense such as texture, taste, as well as odour. Degradability had been shown to affect plant preference or palatability such that the highly degradable plants would be more preferred or favoured (Obour *et al.*, 2015). Plant palatability and degradability decrease as the plant matures. This decrease is due to a decrease in protein while neutral and acid detergent fibres increase, adversely affecting palatability, intake and degradability of substrates (Mpanza, 2015). The proportion and lignification of cell wall also affect plant degradation in the rumen. Plant species with high lignin usually have low degradability values (Mpanza, 2015).

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The low degradability of plant species high in fibre as well as tannins is caused by decreased penetrative capacity to rumen microorganism through lignified plant cell as well as tannins' precipitation influence on microorganism and enzymes (Baloyi *et al.*, 2008). The objective of the study was to assess the nutritive value, *in vitro* dry matter degradability (DMD), intake and relative palatability index of legume residues (*Lablab purpureus and Vigna unguiculata*) and maize straw (*Zea mays*), while hypothesizing that there will a variation on nutritive value, IVDMD, intake and ralative palatability indices of legumes and maize straws.

MATERIALS AND METHODS

The experiment was conducted around December 2020 at the North-West University farm (Mafikeng Campus) (25°49′22″ S and 25°36′54″ E), North-West province of South Africa, with an altitude of about 1290m 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.

Three legumes species used in the experiment were grown under similar soil and management conditions. Three legume species including lablab and two different cowpeas (Betswit and Dr Saunders varieties) were planted as a monoculture in the experimental field, 3 replications for every species. They were assigned to the plots in relation to a randomized complete block design (RCBD). Each block had 3 plots (size of 13 m × 7 m each plot) for each of the 3 different species thus 9 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 90 cm as well as an intra-row spacing of 17 cm in an area of 20 m × 30 m.

The weeding was done when weeds started to germinate. Throughout the growth phase, the crops were inspected frequently for signs of infections as well as pests and remedial action was taken where necessary. Sprinkler performed watering three times a week. Harvesting of maize straws was done at the maturity stage (14 weeks old) and the legume residues at the reproductive stage (13 weeks old). The legumes and maize were harvested from the plots respectively and were left to dry at room temperature for two weeks and ground and place in an airtight container pending chemical analysis. The rest of harvested dried straws and legume residues were cut into small pieces placed in tight bags pending palatability trial around winter season (June 2021).

Both lab work and feeding experiments were run at North-West University experimental farm (Molelwane), Mafikeng (25°47′27″ S and 25°37′18″ E). Samples from each forage were analyzed for laboratory dry matter (DM) as well as ash as stated by AOAC (2005). Neutral detergent fibre (NDF), as well as acid detergent fibre (ADF), was determined by the ANKOM^{2000} Fibre Analyser (ANKOM Technology, New York). Acid detergent lignin (ADL) was determined utilizing H_2SO_4 (72%) as stated by van Soest *et al.* (1991). Total nitrogen (N) was determined by the standard macro Kjeldahl method (AOAC, 2005) and was converted to crude protein (CP) by multiplying the % N content by the factor of 6.25. Ether extract (EE) was determined using the Soxhlet method (AOAC, 2005). Hemicellulose and cellulose were calculated: hemicellulose=NDF-ADF, cellulose=ADF-ADL. Metabolizable energy (ME) was calculated using the formula stated by Khalil *et al.* (1986). The formula applied to estimate total digestible nutrients (TDN) was 82.38 - (0.7515 × ADF) as mentioned by Bath and Marble (1989). Total phenolics were determined using Folin-Ciocalteau 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 methods and expressed as leucocyanidin equivalent (g/kg DM) as stated by Porter *et al.* (1985).

In vitro dry matter degradability of the samples from all the forages was determined using the ANKOM Daisy^{11} Incubator comprising a thermostatic chamber (39°C) with four circling jars according to ANKOM Technology. The samples (0.45-0.5 g) were weighed into ANKOM F57 filter bags and they were heat-sealed and put in the digestion jars. Buffer solutions (2) were prepared and combined at a ratio of 1:5 and 1600 ml of the combined buffers were added to every jar and was warmed at 39°C. Rumen fluid was collected from a fistulated Bonsmara cow (~550 kg). The cow was reared on a mixed diet of Blue buffalo grass as well as Lucerne hay. 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 mutton cloth. Rumen inoculum (400 ml) was added to every jar, every jar contained ANKOM buffer (1600 ml) and F57 bags. To maintain anaerobic conditions, jars were continually purged with CO_2 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 for other withdrawal periods, the bags were washed with distilled water for 20 minutes using ANKOM^{2000} Fiber Analyzer, the bags were then oven dried for 12 h at 105°C earlier being weighed for IVDMD (g/kg).

Five castrated male boer goats (weighing 20.4±3kg) were used in palatability experiment. The goats were housed in a roof and half-walled shed with individually feeding pens. Each goat was provided with four feeding troughs containing different diets. Four feeds (maize straw, lablab, Dr Saunders and Betswit leaves) were utilized. Feeds were used as treatments and goats as replicates in a completely randomized design. The goats were treated against internal parasites before 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. Throughout the data collection period, each goat was offered 0.5 kg of each feed, daily at 08h00. The feed offered was adjusted daily in order to get 10% leftover. The placement order of feeds in the troughs was randomized daily to prevent habit reflex. Animals were given clean water *ad libitum*.

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

T1- Average daily intake of maize.

Ti- Average daily intake of the other feeds,

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

A1- The amount of maize offered.

Ai- The amount of other feeds offered as shown above.

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

P1 = (T1/A1)/(T1/A1) for maize.

Pi = (Ti/Ai)/(T1/A1), where i = 2, 3, or 4.

In this way, Pi was calculated for each feed on daily basis for the period of data collection. For IVDMD and palatability trial, the goats were cared for according to the guidelines provided by Animal Research Ethics Committee of the Institution (North-West University, South Africa) (Ethic Approval No. NWU-02003-20-A5) and of the Federation of Animal Science Societies (FASS) (2010).

Data on chemical composition, IVDMD and the effects of diets on feed intake and relative palatability indices of goats was subjected to a one-way analysis of variance (SAS, 2010). 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.

RESULTS AND DISCUSSION

Nutritive value

The chemical characterization of legumes normally contributes to animal productivity and this nutritive value can assist in assessing the forage quality (Daba *et al.,* 2019). For chemical composition (Table 1), Dr Saunders had the highest (P<0.05) crude protein (CP) content (230.7 g/kg DM). According to Liu *et al.* (2019), crude protein is one of the parameters commonly used for feed quality assessment. The cowpea varieties had a CP content that is more than 15%. These results are in line with the results that were reported by Ravhuhali *et al.* (2010), Washaya *et al.* (2018), Akakpo *et al.* (2020) and Umotoni *et al.* (2021). In the current study, Lablab CP content was similar to the value reported by Washaya *et al.* (2018). Daba *et al.* (2019) explained the importance of protein proportion in low quality diets for ruminants and this contribute to the livestock growth and development; and reproduction. All the legumes in the current study had the CP content higher than the recommended minimum requirement for growth and lactation in ruminants. The high CP of these legumes suggests that they could be used as protein supplements for ruminants fed low-quality roughages. Maize had the least CP content (70 g/kg) and this value may not sufficiently support rumen microorganism activity, thus limiting the amount of protein available to the ruminants (Ravhuhali *et al.,* 2020).

Maize had the highest (P<0.05) NDF content (622.6 g/kg DM) when compared to Dr Saunders (352.0 g/kg DM), lablab (353.5 g/kg DM) and Betswit (394.1 g/kg DM). Betswit had the highest (P<0.05) ADL content than all other forages. The NDF of cowpeas in this study was comparable to the results of Tekle and Gebru (2018). The ADF and NDF of lablab in this study were significantly lower than reported by Seid and Animut (2018) and Jabessa *et al.* (2021). The ADL of lablab in the current was significantly higher than the value reported by Hidosa *et al.* (2018). In the current study, cowpeas had higher ADL and ADF compared to lablab, suggesting that they might be difficult to degrade. This is due to the fact that ADF is primary composed of cellulose and lignin, both of which are not simple degradable (Washaya *et al.,* 2018). Feeds with high lignin typically hamper with fibre availability to ruminants (Roy *et al.*, 2019). Lablab is a legume of choice due to its low ADF content, since high ADF content limit microbial protein synthesis in the rumen (Washaya *et al.*, 2018). The ADL content of maize in the present study was similar to the value obtained by Akinfemi *et al.* (2009). The high fibre fractions of maize straw in the present study may decrease degradability as reported by Rojas-Hernandez *et al.* (2016). This discrepancy among

Table 1: Chemical composition of legumes and maize straws (g/kg DM) unless stated.

Species	DM	Ash	CP	EE	NDF	ADF	ADL	Cel	Hemi	CT	TP	TDN	ME (Mcal/kg)
Maize	915.2^{a}	164.2^{b}	70.6^{d}	19.7^{b}	622.6^{a}	387.6^{a}	141.2^{c}	246.4^{a}	234.9^{a}	0.06^{c}	0.19^{c}	53.3^{d}	2.0^{d}
Dr Saunders	896.8^{c}	136.8^{c}	230.7^{a}	19.5^{b}	352.0^{c}	316.4^{c}	159.6^{b}	156.8^{c}	35.7^{c}	24.0^{b}	0.23^{b}	58.6^{b}	2.26^{b}
Lablab	906.5^{b}	101.8^{d}	222.3^{b}	16.7^{c}	353.5^{c}	302.7^{d}	126.3^{d}	176.5^{b}	50.8^{b}	28.0^{b}	0.36^{a}	59.6^{a}	2.3^{a}
Betswit	896.5^{c}	169.6^{a}	180.7^{c}	34.5^{a}	394.1^{b}	340.3^{b}	176.7^{a}	163.7^{c}	53.8^{b}	50.1^{a}	0.10^{d}	56.8^{c}	2.18^{c}
SE	1.4	1.3	1.1	0.8	2.8	2.4	2.6	2.4	4.0	2.7	0.02	0.18	0.01

^{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; NDF- Neutral detergent fibre; ADF- Acid detergent fibre; ADL- Acid detergent lignin; Cel- Cellulose; Hemi- Hemicellulose; CT- Condensed tannins; TP- Total polyphenols; ME- Metabolisable energy; TDN- Total digestible nutrient; SE- Standard error.

forages could be attributable to differing environmental conditions, sowing season, as well as the time of harvesting as stated by Mbahi *et al.* (2018).

In vitro dry matter degradability

The highest (P<0.05) IVDMD was recorded for lablab and Dr Saunders throughout the withdrawal periods (Fig 1). Based on our findings, in all species there was increasing of substrates degradation throughout the incubation period which were in agreement with the other studies. Ramantsi *et al.* (2019) also reported an increase in IVDMD with increasing incubation time in the leaves. Among legumes, lablab and Dr Saunders had highest degradability values. The higher IVDMD of lablab at 24 up to 72 hrs, could be ascribed to their low lignin and EE content. 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). The low lignin made it easier for microbial enzymes that catalyze their hydrolyses to access these legumes. The Lower IVDMD of Betswit than all other legumes could be attributable to its high ADL, CT as well as EE content. Mudau *et al.* (2021) stressed that high lignin content depresses degradability, since it is indigestible. High EE content causes feed particles to be physically covered limiting microorganism "attack" (Muya *et al.,* 2020). All the legumes performed better than maize, these findings are consistent with the findings of Azim *et al.* (2000), who indicated that legumes have higher degradability than non-legume forages.

Palatability indices

Though all legumes were acceptable and consumed, lablab was much consumed (P<0.05) than any other feed by goats (Table 2). However, goats consumed a similar (P>0.05) quantity of maize and Dr Saunders. Lablab had a higher palatability index (P<0.05) when compared to other diets. The low preference for Betswit in the current study could be ascribed to high CT (50.1 g/kg DM) concentration when compared to other legumes. Deng *et al.* (2017) stated that lower intake and palatability index of *Tararindus indica* was attributable to high CT concentrations when fed to goats. Low concentrations of CT (2-4%) are nutritional beneficial because they reduce rumen protein degradation and increase protein availability for digestion as well as absorption, resulting in good ruminant performance (Ravhuhali, 2018). High concentrations of CT depress feed intake due to astringency. The astringent sensation that goats most likely feel when consuming it may decrease its palatability (Obour *et al.*, 2015, Rojas-Hernandez *et al.*, 2016). The low palatability of Betswit is probably an evolved response by goats to the negative influence of CT on Betswit digestibility (Obour *et al.,* 2015). The low intake of betswit than other legumes was also influenced by high fibre proportions (Lascano *et al.*, 2003).

Regression

In Table 3, from the current study, intake had a strong relationship with palatability. The palatability index was positively and significantly (P<0.05) predicted from the intake of goats (r^2=0.977). There were good and poor associations between the chemical composition of diets and their palatability indices and intakes by goats. The strong intake-palatability relationship is due to the fact that, as the plant grows, there is a saturation of cell wall components together with lignin leading to reduction of CP content. Several researchers reported the relationship between lignin content and palatability (Brown *et al.*, 2016; Abdullah *et al.*, 2017). Even though there are some of the important elements determining palatability such as smell, touch and taste,

Table 2: Feed intake (Ti) and relative palatability index (Ri) by goats.

Species	Intake (kg/d)	Palatability index
Maize	0.27^{b}	1.0^{bc}
Dr Saunders	0.27^{b}	1.07^{b}
Lablab	0.36^{a}	1.42^{a}
Betswit	0.19^{c}	0.78^{c}
S.E	0.02	0.07

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

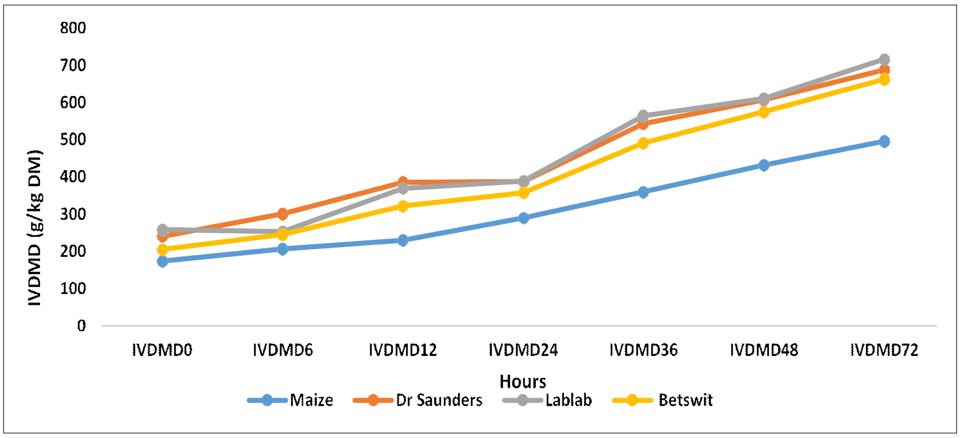

Fig 1: *In vitro* dry matter degradability of legumes and maize straws (g/kg DM) (0, 6, 12, 24, 36, 48 and 72 h).

Table 3: 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

R^2- Regression coefficient.

chemical composition was also regarded as the important factor behind palatability (Brown *et al*., 2016). With the exception of lignin and total phenolics, the results from our study showed that all chemical constituents did not have any relationship with palatability and intake. Ravhuhali (2010) also found no relationship between chemical constituents and palatability indices, while Alanso-Diaz *et al.* (2008) also reported that intake was not associated with CP content, whereas, contrary to our finding Sultan *et al.* (2009) found the positive relationship between CP with palatability indices and intake. The results from our study showed that the highest palatability index was obtained from lablab while, Dr Saunders with high CP content gave medium palatability and intake values in goats. The highest palatability and intake of lablab than other forages could be due to lower lignin content found in this species (Lascano *et al.,* 2003). As the plants advances the lignification on leaves tend to saturated leading to low acceptability by ruminants. 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 Alanso-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.

CONCLUSION

The outcomes from the study showed that the studied legumes had higher CP content making them a good potential for supplementation. Except for phenols, all chemical composition parameters poorly predicted intake and palatability index. However, though having high phenols, lablab can be highly recommended to supplement low-quality roughages such as grass and straws due to high CP content, lower lignin and high degradability. There is a need to assess other phytochemicals present in these legumes and also to run the *in vivo* trial in order to establish the best level of supplementation and the best legume for animal productivity.

Institutional review board statement

The study was conducted according to the guidelines provided by North-West University Research Ethics Committee, with an approval ethic no. NWU-NWU-02003-20-A5.

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Conflicts of interest

The authors declare no conflict of interest.

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