The chemical composition and *in vitro* dry matter digestibility of herbaceous forage legumes adapted to arid Namibia

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Abstract

The nutritional quality of cultivated herbaceous forage legumes adapted to the arid environment of Namibia has not been characterised. These legumes are important protein feed resources for animals. The study was conducted to determine the chemical composition and *in vitro* digestibility of four herbaceous legumes, namely *Tylosema esculentum*, *Vigna unguiculata*, *Lablab purpureus*, and *Mucuna pruriens.* The legumes were harvested at the vegetative growth stage and separated into leaves, stems, and whole plant fractions. The Tylosema had the lowest dry matter (DM) content in the leaf, stem, and whole plant fractions compared to other legumes, which also differed significantly. The ash content of the Vigna leaf and whole plant fractions was higher, 16.93% and 26.32%, respectively, while Lablab had a high ash content in the stem fraction, 10.40%. Tylosema had the lowest ash content among legumes in all fractions, ranging from 4.81% to 7.87%. Tylosema had the highest organic matter (OM) values, ranging from 92.13% to 94.20%. Lablab had the highest crude protein (CP) in all plant fractions, ranging from 12.95% to 30.05%. The legumes had similar hemicellulose content in leaf and whole plant fractions, while the stem fraction of Vigna had the highest hemicellulose content. The legumes had significantly different *in vitro* DM digestibility. Vigna recorded the highest DM digestibility in the leaf (88.83%) and stem (74.93%) fractions, while Lablab recorded the highest digestibility in the whole fraction (84.53%). In conclusion, forage legumes differed in chemical composition and *in vitro* DM digestibility, but all had adequate protein above the minimum requirement of 7% for ruminant livestock diets.

Keywords: Environment, hemicellulose, nutritional quality, plant fractions

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1. Introduction

Rangelands, primarily covered by natural vegetation comprising both grass and browse species, provide grazing and forage for livestock and wildlife. Namibia is an extensive livestock production country with cattle, sheep, and goats relying heavily on rangelands. Developing countries, including Namibia, experience feed shortages both in terms of quantity and quality, mainly protein, particularly during the dry season. Alternate feed resources, such as adapted arid forage legumes, could play an important role in mitigating the shortage of high-quality feed (Kamati et al., 2019). In Namibian rangelands, there is a wide range of forage legume species that can be valuable sources of protein for ruminants (Kaholongo, 2016). Arid adapted herbaceous legumes have higher crude protein content compared to rangeland grasses, which contribute to dietary diversity and supply the required protein to meet nutrient requirements that support livestock growth and productivity. Climate change is anticipated to immensely threaten southern Africa, and agriculture would suffer the most (Minwuye, 2017; Tendonken *et al*., 2022). However, there are tropical legumes that have survived under harsh environmental conditions and contribute to the diet of livestock as protein supplements especially during the dry season (Washaya et al., 2025) (Madibela, 2018). To survive the arid environments, most forage legumes have developed morphological and physiological mechanisms (Adeboye, 2017). The presence of the forage legumes provides a high-quality diet for livestock, because legumes have a higher protein content than grasses at the mature growth stage (Kebede et al., 2016; Mariyappan, 2009). Currently, there is limited information on the nutritional value of leguminous forages such as Marama, Velvet bean, Cowpea, and Lablab that can be grown in the Namibian arid environments. Such legumes are among the few climate-smart forage legumes that are adapted and can be grown in arid and semi-arid environments as potential livestock protein supplements feeds. Therefore, the study was conducted to evaluate the chemical composition and *in vitro* dry matter digestibility of plant fractions of four cultivated herbaceous legumes, namely *Tylosema esculentum* (Marama)*, Vigna unguiculata* (Cow pea), *Lablab purpureus* (Lablab), and *Mucuna pruriens* (Velvet beans).

2. Materials and Methods

2.1. Description of study site

The study was conducted at the University of Namibia, Neudamm Campus, which is located at 22°30’07” S and 17°22’14” E, and an altitude of 1762 meters above sea level. The farm is in the Khomas Highland savanna, with average day temperatures of 30°C in January to 20°C in July, while the night temperatures range between 17°C in January and 7°C in June (Mendelsohn, 2002) to less than –1°C in winter. The area receives an average annual rainfall ranging between 350 and 400mm, with most of the rainfall received from January to April (Mendelsohn, 2002). The soils are dominated by homogeneous Lithic Leptosols and Eutric Regosols soil types (Geological Survey of Namibia, 2011). The soils are rich in materials derived from physical weathering and are suitable for the growth of forage legumes such as Marama plant. The vegetation is characterised by woody species such as *Senegalia mellifera, Vachellia hereroensis, Vachellia hebeclada, Vachellia reficiens, Euclea undulata, Dombeya rotundifolia, Tarchonanthus camphoratus, Rhus marlothii, Albizia anthelmintica,* and *Ozoroa crassinervia* and various grass species such as *Cenchrus ciliaris*, *Anthephora pubescens*, *Eragrostis*, and *Aristida* species (Karuaera, 2011).

2.2. Forage harvesting and sample preparation

Four leguminous species, namely *Tylosema esculentum* (Marama)*, Vigna unguiculata* (Cow pea), *Lablab purpureus* (Lablab) and *Mucuna pruriens* (Velvet beans) were manually harvested at the vegetative growth stage at Neudamm Farm Research field. The harvested legume forages were separated into leaf fraction, stem fraction, and whole fraction and shade dried to a constant weight. After drying, the plant fractions were milled through a 1mm screen, and the milled samples were stored in airtight containers pending laboratory analysis.

2.3. Chemical analysis

The milled legume plant fractions were analysed for dry matter (DM) content by drying the samples in a forced draught oven at 100 °C for 24 h (AOAC, 2000). The ash content was determined by incineration in a muffle furnace at 550 °C for 6 h (AOAC, 2000). The crude protein (CP) content was determined using the AOAC method no. 978.04 (AOAC, 2000), which determined the total nitrogen (N) content of the samples and CP obtained by multiplying the percentage of N content by a factor of 6.25. The crude ether extract (EE) was determined using the AOAC method 920.39 (AOAC, 2000). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the samples were determined following the procedures of Goering and Van Soest (1970), using an ANKOM fibre analyser (ANKOM Technology, Macedon, NY, USA). The hemicellulose content of the plant fractions was determined as the difference between NDF and ADF (Rinne *et al*., 1997).

The dry matter *in vitro* digestibility of the legume plant fractions was determined using Daisy II incubator (ANKOM Technology Corp, USA). Rumen fluid was collected from a slaughtered steer (Fortina et al., 2022) that was fed with a diet of grass hay and lucerne hay *ad libitum*. Samples of 0.5 g were weighed into ANKOM F57 bags, heated-sealed, and incubated at 39 ^{0}C for a period of 48 hours in a ruminal fluid which was combined with a buffer solution at a ratio of 1:4 (v/v) in a Daisy^{II} incubator jar. After incubation, the jars were drained, and the bags were thoroughly rinsed under running tap water and oven dried at 60 °C for 48h and weighed. The *in vitro* dry matter digestibility of the leguminous forage plant fractions was then calculated from the dry matter placed in the bag and the dry matter residues remaining in the bag.

2.4. Statistical analysis

The chemical composition and *in vitro* DM digestibility data were analysed using a one-way analysis of variance (ANOVA) in the Statistical Package for Social Sciences (SPSS version 27) (IBM, 2020) with the hypotheses being tested at 5% level of significance. The differences between means were tested using the Duncan Multiple Range Test.

The statistical model employed for data analysis is given in the equation below:

$$\boldsymbol{Y_{ij}}=\mu+\boldsymbol{\tau_i}+\boldsymbol{\varepsilon}_{ij} \qquad (1)$$

Where:

- Y_{ij}= the dependent variable (e.g., CP, NDF content, or *in vitro* DM digestibility measurement for the j^{th} replicate of the i^{th} herbaceous legume). (i=1, 2, 3, 4 and j=1, 2, 3, 4)
- μ= the overall experimental mean.
- τ_i= the effect of the i^{th} legume (i=1, 2, 3, 4)

ε_{ij}= the random error of the j^{th} replicate of the i^{th} legume

3. Results

3.1. Chemical composition of the forage legumes leaf, stem, and whole fractions

Chemical composition of legume leaf fraction varied significantly (P < 0.05) except for hemicellulose (Table 1). The dry matter content was highest in Lablab (94.28%), followed by Velvet beans and Cowpea (93.83% and 93.70%, respectively), and lowest in Marama leaves (90.52%). Marama leaves had the highest OM content (94.20%), followed by Velvet beans (90.00%), then Lablab with a content of 89.33% while Cowpea had the least of 83.07%. Among the legumes, the crude fat content of Lablab was the highest (P < 0.05), followed by Marama and Cowpea, which were intermediate and similar, while Velvet bean had the lowest mean values. The CP content of Lablab leaves was the highest (P < 0.05) CP with a mean value of 30.05 % followed by Cowpea, Velvet beans, and Marama, which also differed (P < 0.05) among themselves with values of 26.70%, 22.70% and 16.95%, respectively. The NDF content was highest in Marama leaves (34.12%) compared to other legumes. Moreover, Marama leaves had the highest (32.46%) ADF content (P < 0.05) while Cowpea and Velvet beans had intermediate values (25.87 and 24.19%) and Lablab had the lowest ADF content (21.77%). The leaf fraction of the four legumes was similar ($P > 0.05$) in their hemicellulose content.

Table 1. Chemical composition of the leaf fractions of four herbaceous legume forages

Nutrient (%)	**Cowpea**	**Lablab**	**Marama**	**Velvet beans**	**SEM**	**P-value**
Dry matter	93.70^{b}	94.28^{a}	90.52^{c}	93.83^{b}	0.07	0.002
Ash	16.93^{a}	10.67^{b}	7.87^{d}	10.00^{c}	0.07	0.003
OM	83.07^{d}	89.33^{c}	94.20^{a}	90.00^{b}	0.07	0.001
Crude protein	26.70^{b}	30.05^{a}	16.95^{d}	22.70^{c}	0.41	0.004
Crude fat	10.19^{b}	18.42^{a}	9.71^{b}	4.23^{c}	0.78	0.003
NDF	28.89^{b}	23.34^{b}	34.12^{a}	26.98^{b}	1.26	0.001
ADF	25.87^{b}	21.77^{c}	32.46^{a}	24.19^{b}	1.22	0.002
Hemicellulose	3.02	1.58	2.16	2.79	1.70	0.760

^{abcd} Means in the same row with different superscripts are significantly different ($P < 0.05$)
OM= Organic matter; NDF= Neutral detergent fibre; ADF= Acid detergent fibre

The chemical composition of the stem fractions of the four forage legumes was significantly ($P < 0.05$) different (Table 2). The stem fraction of Velvet bean had the highest DM content (94.38%), followed by Cowpea (94.11%), then Lablab (93.91%), and least was Marama (89.77%). The legume stem fractions had different ($P < 0.05$) ash content, with Lablab having the highest mean value of 10.40% while Marama had the least (4.81%). The stem fractions of Marama and Velvet bean had the highest ($P < 0.05$) OM content compared to Cowpea and Lablab, which also differed ($P < 0.05$). The Lablab stem fraction had the highest CP content ($P < 0.05$), followed by Velvet bean, which was intermediate, and the least was Cowpea and Marama, which were similar ($P > 0.05$). Among the legumes, the stem fraction of Lablab had significantly higher ($P < 0.05$) fat content (18.64%) compared to Cowpea, Marama, and Velvet beans, which had similar ($P > 0.05$) fat content. Cowpea and Lablab had the lowest NDF content, while Marama was intermediate, and Velvet bean had the highest NDF content. Marama and Velvet bean had the highest ADF content, while Velvet bean and Lablab were intermediate and similar, and Cowpea had the least ADF content. The hemicellulose was greatest in Cowpea, followed by Lablab and Velvet bean, which were similar ($P > 0.05$), and Marama had the least.

Table 2. Chemical composition in the stem fraction of the four herbaceous legume forages

Nutrient (%)	**Cowpea**	**Lablab**	**Marama**	**Velvet beans**	**SEM**	**P-value**
Dry matter	94.11^{b}	93.91^{c}	89.77^{d}	94.38^{a}	0.04	0.003
Ash	8.26^{b}	10.40^{a}	4.81^{d}	7.33^{c}	0.17	0.002
Organic matter	91.74^{b}	89.60^{c}	92.13^{ab}	92.67^{a}	0.17	0.004
Crude protein	9.18^{c}	12.95^{a}	9.53^{c}	10.15^{b}	0.13	0.003
Crude fat%	11.64^{b}	18.64^{a}	7.02^{b}	5.63^{b}	1.54	0.001
NDF	50.05^{c}	51.02^{c}	52.16^{b}	54.02^{a}	1.60	0.03
ADF	38.11^{c}	44.84^{b}	50.65^{a}	46.26^{ab}	1.64	0.002
Hemicellulose	11.93^{a}	6.18^{b}	1.51^{c}	7.76^{b}	1.15	0.007

^{abcd} Means that in the same row with different superscripts are significantly different ($P < 0.05$). OM = Organic matter; NDF = Neutral detergent fibre; ADF = Acid detergent fibre.

The whole plant fraction chemical composition of Lablab, Velvet beans, Marama, and Cowpea is shown in Table 3. The whole plant fraction of Marama had the lowest ($P < 0.05$) DM content, while the other three legumes had similar ($P > 0.05$) DM content. Cowpea whole plant fraction had the highest ($P < 0.05$) ash content, while Velvet bean had the least content among the legumes. There was a significant ($P < 0.05$) difference in the OM values of the four legume whole plant fractions, with Marama having the highest OM content and Cowpea with the least content. Among the legumes, Lablab whole plant had the highest CP (20.35%), followed by Cowpea (16.35%), then Velvet bean (13.80%), while Marama had the least CP (11.80%) content. Marama and Cowpea whole plant fractions had significantly ($P < 0.05$) the highest crude fat content compared to Lablab and Velvet bean, which did not differ ($P > 0.05$) between themselves. The NDF and hemicellulose mean values of the four legumes were not different ($P > 0.05$). However, the ADF content varied ($P < 0.05$) among the legumes, with Marama having the highest content compared to the other three legumes, which were similar ($P > 0.05$).

Table 3. Chemical composition of whole plant of the four herbaceous legume forages

Nutrient (%)	Cowpea	Lablab	Marama	Velvet beans	SEM	p-value
Dry matter	94.22^{a}	94.14^{a}	90.20^{b}	94.06^{a}	0.08	0.006
Ash	26.32^{a}	10.65^{b}	5.81^{d}	8.43^{c}	0.37	0.004
OM	62.27^{d}	89.36^{c}	94.19^{a}	91.57^{b}	0.37	0.003
Crude protein	16.35^{b}	20.35^{a}	11.80^{d}	13.80^{c}	0.49	0.005
Crude fat	7.58^{a}	5.47^{b}	9.51^{a}	5.25^{b}	0.69	0.03
NDF	47.95	38.51	46.86	36.17	6.71	0.590
ADF	34.18^{b}	33.50^{b}	40.45^{a}	31.49^{b}	4.85	0.003
Hemicellulose	13.77	5.01	6.41	5.14	6.60	0.650

^{abcd} Means in the same row with different superscripts are significantly different ($P < 0.05$).
OM = Organic matter; NDF = Neutral detergent fibre; ADF = Acid detergent fibre.

3.2. *In vitro* dry matter digestibility of the forage legume plant fractions

The *in vitro* DM digestibility of Lablab, Velvet bean, Marama, and Cowpea leaf, stem, and whole plant fractions is shown in Table 4. In the leaf fraction, the *in vitro* DM digestibility differed ($P < 0.05$) among the legumes. Cowpea leaf fraction had the highest in vitro DM digestibility (88.83%), followed by Lablab (85.06%), then Velvet bean (76.43%), while Marama recorded the least (69.60%). The stem fraction DM digestibility also differed among the four legumes. Cowpea and Lablab had the highest DM digestibility compared to Marama and Velvet bean, which were similar ($P > 0.05$). However, the DM digestibility did not differ between Lablab, Marama, and Velvet bean. In the whole plant fractions, Lablab had the highest ($P < 0.05$) DM digestibility (84.53%), followed by Cowpea, which was intermediate, while Marama and Velvet bean had the least DM digestibility values, which were similar ($P > 0.05$).

Table 4: The *In vitro* DM digestibility of plant fractions of the herbaceous legumes

Plant fraction	Cowpea	Lablab	Marama	Velvet beans	SEM	P-value
Leaf fraction	88.83^{a}	85.06^{b}	69.60^{d}	76.43^{c}	0.51	0.002
Stem fraction	74.93^{a}	68.69^{ab}	62.25^{b}	61.08^{b}	2.42	0.005
Whole plant	72.72^{b}	84.53^{a}	62.64^{c}	66.16^{c}	1.54	0.003

^{abcd} Means in the same row with different superscripts are significantly different ($P < 0.05$)

4. Discussion

4.1. Chemical composition of the forage legumes

The nutritive value of forages depends on the nutrient content, voluntary intake, and digestibility of the feed. The dry matter is an indicator of the amount of nutrients that are available to the animal in a ration (Saha *et al*., 2017). Livestock consume a certain amount of dry matter per day (kg/day) to meet their daily nutrient requirements for maintenance and production. The overall DM contents of the forage legumes' whole plant fractions ranged from 90.20 to 94.28% which was higher than the values reported by Washaya (2018) of 92.3% and 92.72%, for Cowpea and Lablab, respectively. The differences between the current study values compared to literature values could be due to the stage of growth when the forages were harvested, species, and differences in the soil fertility in which the legumes were grown. It is reported in the literature that the nutrient content of forage legumes changes continuously with the growth and development of the plant, hence, the stage of maturity significantly affects chemical composition (Norton and Poppi, 1995; Adesogan *et al*., 2009). The DM content of Cowpea and Lablab was similar, and in agreement with the results reported by Ayana *et al.* (2013).
Cowpea had a high ash content in the leaf and whole plant fraction compared to other legumes, while Lablab recorded high ash in the stem fraction. Ash is the indicator of inorganic residue, which provides a measure of the total amount of minerals within a particular feed. Lablab recorded similar ash percentages to those recorded by Washaya (2018). Marama recorded the least ash content in all fractions, ranging from 4.81-7.87%. Cowpea recorded a higher ash content in the leaf fraction (16.93%) than that reported by Kamati (2016) of 12.15-12.06%. Marama forages have a significantly high organic matter content compared to Cowpea, Lablab, and Velvet bean forages. This could be because of the relatively higher drought tolerance of Marama, by storing all its glucose and starch to tolerate drought (Cullis, 2018). The organic matter content of Velvet bean forages is similar to values reported by Martinez-Perez (2018).

In the current study, the CP content of the whole plant fractions ranged from 9 to12%, and this was lower than the CP content reported by Norton and Poppi (1995) and Mupangwa (2000), who indicated that forage legumes generally have a CP range of 14 – 20%. Lablab recorded the highest CP in all three fractions, and this was probably because Lablab had low NDF and ADF in all three fractions. The CP content of the leaf was significantly higher, ranging from 22-30% compared to stem and whole plant fractions, and this could be attributed to low NDF and ADF in the leaves. These results are comparable to those reported by Murphy (1999). For animals grazing forages, dietary CP concentrations exceeding 20% of DM exceed requirements, even for lactating cows (Pacheco and Waghorn, 2008). This therefore suggests that the leaf fractions of the legumes supply excess dietary CP, which has significant metabolic costs for the disposal of excess N in the form of urea in urine (Pacheco and Waghorn, 2008). Excess dietary protein is converted to either urea in the liver or nitrous oxide (Tan and Murphy, 2004) from the urine resulting in emission of a greenhouse gas (Rojas-Downing *et al*., 2017).

The results show an overall high NDF and ADF in the stem fraction of the forage legumes compared to the leaf and whole plant fractions. This is probably because the stem fraction accumulates structural carbohydrates as the plant matures. In forages, NDF is a measure of the proportion of cell wall, which consists of hemicellulose, cellulose, and lignin. In a ruminant diet, NDF is required as it enables proper rumen function and rumen mat formation. However, increasing NDF in the diet may lead to excessive gut fill and limit feed intake. Acid detergent fibre is a measure of cellulose and lignin, with cellulose digestibility varying depending on lignin content. As the lignin increases in the forage, the digestibility of the forage decreases.

The low values of ADF recorded with Cowpea, Lablab, and Velvet beans in leaves and whole plant fractions make them legumes of choice since low values of ADF imply that the forage fractions are highly degradable in the rumen (Ayan *et al.,* 2012). Forage legumes accumulate structural carbohydrates as the plant matures, and the stem matures first than the leaves, resulting in high NDF and ADF compared to the leaf fraction. That is due to lignification, which translates into low degradability within the rumen. The NDF content of legume forages has been the major drawback to their full utilisation in animal nutrition (Washaya, 2018). The variation in NDF concentration could also be due to differences in the cell-wall structure of herbaceous legumes (IAEA, 2006). Cowpea had a generally high hemicellulose in all three fractions compared to the other forages. Amasaib (2021) reported that the high hemicellulose content of forage legumes is linked to higher degradability.

4.2. *In vitro* DM digestibility of forage legumes

The digestibility of a feed determines the availability of feed nutrients to the animal (Hidosa, 2017). Lablab and Cowpea recorded high *in vitro* DM digestibility in leaves and stems fractions compared to Marama and Velvet beans forages in the same fractions. Generally, forage legumes have a high digestibility due to their low NDF and ADF content compared to grasses. *In vitro* DM digestibility (IVDMD) results vary due to the method of evaluation, genotypes and climatic environments, and plant part (Solomon and Kibrom, 2014).

The supplementation of animals that are fed on poor-quality feed with forage legumes improves the digestibility of feed by the ruminants (Hidosa, 2017). The relatively low content of fibre and high crude protein content can facilitate the colonisation of the feed by the rumen microbial population, which in turn might induce higher fermentation rates, therefore improving digestibility (Van Soest, 1994). Lablab recorded higher IVDMD than what was reported by Murphy (1999), which were 64.6%-71.6% in leaf fraction, 51.6%-61.1% in stem fraction, and 65.6% to 71.6% in whole plant fractions. Dry matter digestibility is limited by the stage of growth of forages (Mupangwa *et al*., 2006; Ravhuhali *et al*., 2010), which is attributed to the lignification of plants as they mature. High fibre diets would require fibrolytic protozoa and fungi rather than cellulolytic bacteria to achieve enough fermentation (Yaynashet *et al.,* 2009).

5. Conclusion

The four herbaceous forage legumes varied greatly in chemical composition as well as in the *in vitro* DM digestibility of their plant fractions. Lablab consistently had the highest CP content in all plant fractions among the four legumes, while Marama had the lowest CP content. In conserving these forage legumes as hay, there is a need to avoid leaf shatter, as it will result in a low-quality hay because the leaf fraction has higher CP content than the stem fraction. The legumes all had adequate protein above the minimum requirement of 7% in ruminant livestock diets, thus making them potential protein supplements to livestock grazing on the arid Namibian rangelands.

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