

AN EVALUATION OF THE POTENTIAL OF SELECTED INDIGENOUS NAMIBIAN  
FORAGE LEGUMES FOR FEEDING GOATS

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## ABSTRACT

The objectives of this research were to determine the chemical composition, palatability, voluntary feed intake (VFI) and effects on body weights of Boer goats fed native forage legumes compared to lucerne and lablab. In experiment 1, the chemical composition of 14 legumes was determined. In experiment 2, the palatability of *Crotalaria argyraea*, *C. podocarpa*, *Otoptera burchellii* and *Vigna lobatifolia* was determined relative to Lucerne in a cafeteria feeding system over a 10-day period. In experiment 3 on VFI, 32 goats were randomly allocated to nine treatments with the legumes *Lablab purpureus* (LP), *O. burchellii* (OB), *V. lobatifolia* (VL) and *Medicago sativa* (LC) as follows: control (C); 18% inclusion (LP18, OB18, VL18 and LC18); and 31% inclusion (LP31, OB31, VL31 and LC31). All forage legumes except *Meulobium candicans* had CP  $\geq$  15%; the NDF (%) ranged from  $30.3 \pm 3.8$  (*C. heidmannii*) to  $56.1 \pm 3.2$  (*L. purpureus*) subsp. *Uncinatus* verde.var. *rhomboideus*. All the four legumes in experiment 2 were highly palatable relative to Lucerne, but a 5-day adaptation period was required for the *Crotalaria* species. Least squares means of VFI (g DM/day) were:  $668.7 \pm 8.8$  (C),  $821.2 \pm 10.8$  (LP18),  $783.1 \pm 9.9$  (OB18),  $777.7 \pm 10.8$  (VL18),  $802.8 \pm 10.8$  (LC18),  $994.4 \pm 9.9$  (LP31),  $992.8 \pm 10.8$  (OB31),  $1009.2 \pm 10.8$  (VL31) and  $970.6 \pm 10.8$  (LC31). Legume supplementation of basal grass hay increased VFI at 31% compared to 18% inclusion level. Body weights were affected ( $P < 0.05$ ) by treatment; the least squares means (kg) for C, LP18, OB18, VL18, LC18, LP31 OB31, VL31 and LC31 were:  $19.2 \pm 0.8$ ,  $22.1 \pm 0.9$ ,  $17.6 \pm 0.9$ ,  $18.1 \pm 0.9$ ,  $16.4 \pm 0.9$ ,  $18.9 \pm 0.8$ ,  $16.6 \pm 0.9$ ,  $21.1 \pm 0.9$  and  $20.4 \pm 0.9$ , respectively. Native forage legumes have the potential to improve nutrition of goats.

**Key words:** native forages, forage legumes, voluntary feed intake, palatability, supplementation

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## **DEDICATION**

I would like to dedicate this thesis work to my God Almighty “Yaweh-Rohi” for His grace and mercy and for giving me the strength, wisdom and knowledge. I would also like to dedicate it to our daughter Mina and son Jeffrey.

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**DECLARATION**

I, Diina Nandjaba Thamina, hereby declare that this study is a true reflection of my own research, and that this work has not been submitted for a degree in any other institution of higher education.

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Date: 09 April 2018

Diina N. Thamina

## CHAPTER 1

### 1. INTRODUCTION

#### 1.1 Background information

Namibia is the driest country in Sub-Saharan Africa (SSA) with harsh climatic conditions characterized by inadequate, late and erratic rainfall in several parts of the country and this affects both crop and livestock production. Severe and prolonged drought conditions for instance as occurred in the years 2013 to 2015, result in poor pastures or rangelands with less palatable grass and legume species. The fluctuation of nutrient content of natural pastures and availability of forage from year to year and between the wet (November/December to March/April) and dry (May to October) seasons compromise livestock farming. In addition, poor management of rangelands, seasonal variability and climate change are a serious threat to the genetic resources and biodiversity of palatable forage legume species (Mpanza et al., 2014).

About 70% of the Namibian population derives part of or its entire livelihood from agriculture directly or indirectly through employment and income generation (MAWF, 2015). Agriculture in Namibia plays a fundamental role to the country's economic development. According to the latest statistics (MAWF, 2015), the agricultural sector and its related industries contributed over 3.7% to the Gross Domestic Product (GDP) in 2014, of which 60% is attributable to the livestock sub-sector. The Namibia Census of Agriculture (NCA) of 2013/2014 reported that 39 percent out of 159, 484 agricultural households in communal areas, are engaged in livestock farming (NSA, 2015). Factors contributing to low agricultural production in Namibia include low and erratic rainfall, extreme water scarcity, insufficient productive land, low soil fertility, overgrazing and bush encroachment.

About 44% of the Namibia's land area is devoted to large-scale "commercial farming" on freehold land; grazing areas in the communal areas constitute 41%, the remaining 15% are state lands including conservation areas (Sweet, 1998). Red meat produced on the freehold land is primarily for export, in addition to supplying domestic consumers (Kroll and Kruger, 1998). The goals of communal farmers on the other hand are diverse including food production for personal use, some marketable surplus and maintenance of high stock numbers. Maintaining high stock numbers represents capital accumulation, enhances social status, helps meet obligations, minimizes production risk because large herds can be split and kept at different localities, hence reducing chances of a total loss (Kroll and Kruger, 1998). This nonetheless leads to the carrying capacity being exceeded leading to overgrazing and rangeland degradation, hence unsustainability.

Inadequate supply and poor quality of the available energy and protein feed stuffs, particularly during the dry season, is a major constraint to livestock production in the tropics and sub-Saharan Africa and this has been the reason for poor performance (Richardson and Smith, 2006; Jamala et al., 2013). Additionally, conventional feeds such as grain and oilseed cake in the tropical regions are not produced in sufficient quantities to meet the needs of both man and livestock (Abule et al., 2005a). Beside harsh climatic conditions, overstocking also attribute to poor livestock performance. Overstocking is a common predicament in all the communal areas of Namibia. In most cases the number of livestock exceeds the carrying capacity of the land putting more pressure on vegetation which leads to overgrazing, soil erosion, reduced soil fertility and in the long run it may result in desertification.

Subsequently livestock off-take rate from communal sectors is lower than that of the commercial farming systems. The livestock off-take in commercial farms is about 27.5% (Barnes et al., 2012) in contrast, in communal areas mostly under extensive farming system, the off-take rate is as low as 5 – 8% (Deloitte, 2013). The lower off-take from the communal areas is mainly attributed to low fertility and high mortality rates. The calving and kidding rates in communal areas range from 35 to 60 % depending on the region; in commercial farms it is estimated to be 65% (Barnes et al., 2012). Meanwhile mortality rate for small stock was estimated to be 16.5% in commercial farms and 23% in communal areas (Barnes et al., 2012).

Natural pastures are the predominant form of grazing for ruminants, but their nutritive values can drop during the dry season, reducing digestibility and animal performance. The predominant source of crude protein (CP) in Namibia's natural pasture are shrubs (e.g. *Lycium oxycarpum*, *Acacia nebrownii*) and trees (e.g. *Acacia erioloba*, *Albizia anthelmintica*); although a number of grasses have moderate to high (10-20%) levels of CP when they are immature (Wesuls et al., 2009). There is a decline of crude protein content in Namibian pastures to below 7% during the dry months of May to November and this can reduce feed intake (Wesuls et al., 2009).

During such periods, livestock performance is adversely affected because animals are unable to consume sufficient nutrients from available forages in order to meet their requirements and maintain satisfactory production levels. The level of CP in pasture also decreases gradually with increasing maturity; similarly fiber concentration increases with maturity. In order to achieve acceptable levels of animal production, protein supplement feeding is indispensable, although it is expensive.

Namibian rangelands have a wide of naturally occurring forage legume species that have the potential to alleviate some of the feed scarcity and nutritional deficiencies and at the same time reduce costs involved in purchasing commercial supplements. The costs of commercial legume forages supplements such as Lucerne (*Medicago sativa*) and concentrates usually escalate during the months of May to November and most farmers especially communal farmers cannot afford to pay such prices. The shortage of good-quality feeds and increased cost of commercial protein supplements necessitated investigation of the potential of indigenous forage legumes as feed supplements for ruminants in Namibia during the dry season.

Indigenous legumes are well adapted to the harsh environmental conditions in Namibia. They can survive even under low and irregular rainfall because they are likely to have deep roots systems (Megan, 2003). They are also adapted to survive under low P soils (Suriyagoda et al., 2010). MAWF (1997) recognized the shortcomings of introduced exotic forage legumes and recommended that future research should rather concentrate on native forage legumes, but to the best of our knowledge there has not been any such undertaking yet. Hence, this study was designed to explore the potential of indigenous forage legumes in Namibian rangelands, as supplementary feed to improve livestock productivity.

## **1.2 Statement of the problem**

The basic reason for poor performance of livestock in developing countries is the seasonal shortage of feed, both in quantity and quality (Richardson and Smith, 2006). There is a decline of crude protein (CP) content in Namibian pastures to below 7% during the dry months of May to November (Wesuls et al., 2009). Namibian rangelands have a variety of naturally occurring forage legume species that have the potential to alleviate the feed scarcity and nutritional deficiencies. Indigenous legumes can be used to supplement low quality grass hay and

crop residues, which in turn may improve nutrition of ruminants. To the best of our knowledge there is no published information on the nutritive value of indigenous forage legumes in Namibia rangelands and it is conceivable they are well adapted to the harsh environment, may contain sufficient CP, are highly palatable, and yet, still overlooked in terms of contributing to ruminant nutrition. Therefore, this study was conducted to determine the nutritive values of native forage legume species by evaluating their chemical composition, palatability and voluntary feed intake.

### 1.3 Objectives of the study

The overall objective of this study was to explore the potential of indigenous forage legumes in Namibian rangelands, which have hitherto been ignored, so as to increase livestock productivity.

The specific objectives of this study were to:

- (i) Determine the chemical composition of twelve identified indigenous and two improved (lablab [*Lablab purpureus*] and Lucerne or alfalfa [*Medicago sativa*]) forage legumes;
- (ii) Determine the palatability of four (4) selected indigenous forage legumes namely *Crotalaria argyrea*, *Crotalaria podocarpa*, *Otoptera burchellii* and *Vigna lobatifolia*, relative to Lucerne (*Medicago sativa*);
- (iii) Determine the voluntary feed intake of weaned Boer goats fed grass hay supplemented with two (2) indigenous forage legumes (*Otoptera burchellii* and *Vigna lobatifolia*) and two (2) improved forage legumes (*Medicago sativa* and *Lablab purpureus*) at two (2) inclusion levels (18% and 31%);
- (iv) Determine the effect of grass hay supplemented with two (2) indigenous forage legumes (*Otoptera burchellii* and *Vigna lobatifolia*) and two (2) improved forage legumes



(*Medicago sativa* and *Lablab purpureus*) at two (2) inclusion levels (18% and 31%) on ADG and body weights of weaned Boer goats;

#### **1.4 Hypotheses of the study**

H<sub>01</sub>: There are no significant differences in chemical composition of the fourteen (4) forage legumes;

H<sub>02</sub>: There are no significant differences in relative palatability of the four selected forage legumes (*Crotalaria argyraea*, *Crotalaria podocarpa*, *Otoptera burchelli*, *Vigna lobatifolia*) and lucerne;

H<sub>03</sub>: The voluntary feed intake of weaned Boer goats fed basal grass hay supplemented with the four (4) legumes (*Lablab purpureus*, *Otoptera burchellii*, *Vigna lobatifolia* and lucerne) at two inclusion levels (18% and 31%) is similar.

H<sub>04</sub>: The average daily gain (ADG) and body weights of weaned Boer goats fed basal grass hay supplemented with the four (4) legumes (*Lablab purpureus*, *Otoptera burchellii*, *Vigna lobatifolia* and lucerne) at two inclusion levels (18% and 31%) is similar.

#### **1.5 Significance of the study**

The results of this study could provide alternative protein supplements for ruminants which are locally available. Indigenous forage legumes can also help to reduce the costs associated with buying concentrates and supplements. This study will provide documentation and as well contribute to sensitization on the need for conservation of desirable indigenous forage legume species existing in Namibian rangelands. The results would also provide information on alternative feed ingredients for the Namibian feedlots.

## 1.6 Limitations of the study

1. Could not assay for anti-nutrients.
2. Limited scope of legumes investigated. Only a limited number of legumes were assayed for chemical composition; moreover most legumes were available in limited quantities, hence palatability and voluntary feed intake could not be done except on a selected few. Also in vitro digestion trials were not done due to time constraints, hence future studies should investigate these parameters so as to narrow down the range of those that can be investigated further in agronomic trials for different agro-ecologic zones.
3. Lack of estimates for digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and Neutral detergent fibre (NDF).
4. Fluctuations in quality of grass hay. The experiment on voluntary feed intake was done in 2015 when there was a severe drought and grass hay had to be bought from commercial suppliers whose quality of the grass hay fluctuated widely for different batches. Although repeated measures analysis using Proc Mixed (SAS, 2008) enabled disentanglement of confounding effects due to fluctuations in quality of basal grass hay, some treatment effects may not have been detected or may have been imprecisely determined.
5. Restriction on performance parameters investigated. Given that this study is the first of its kind, the nutritive value of indigenous forage legumes as supplements was restricted to one component of productivity, viz growth. Other parameters such as carcass quality, conception rates and milk yield and quality could be useful aspects in evaluation of native forage legume supplements.

## CHAPTER 2

### 2 LITERATURE REVIEW

#### 2.1 Livestock nutrition challenges in marginal lands

Most rangelands in Namibia comprise of both grass and browse species and as a result ruminants usually consume a mixture of forage plants specifically grass, shrubs and trees. These components of pasture are appropriate for goats since they are browsers. Their grazing or browsing behavior allows them to maneuver their lips to avoid eating stems and select only the highest quality forage (Poore and Luginbuhl, 2002). Accordingly, they can utilize plants that other domestic ruminant species would not consume.

Sustainable livestock production usually entails efficient use of available feed resources. One of the main constraints to livestock productivity in the tropical regions is poor nutrition caused by inadequate quantity and quality of feed (Kaitho et al., 1997; Alemu et al., 2014; Aganga and Tshwenyane, 2003). This is most pronounced during the dry months of the year when the quantity and quality of the forage available drastically declines and may not meet maintenance requirement of grazing animals.

Severe and prolonged drought conditions which are not infrequent also result in poor pastures or rangelands with less palatable grass and legume species. Poor rangeland management and the desire to constantly increase herd sizes have contributed to overstocking in the communal areas. Moreover the absence of land tenure means there is no incentive for making investments for rangeland improvements in communally owned land, leading to overgrazing. This situation has been exacerbated by farmers with huge numbers of livestock who tend to illegally fence-off large portions of communal land, leaving other farmers with little grazing land

(Kohima and Mandimika, 2016; Muduva, 2014) and as a result causing overgrazing. Unrelenting overgrazing does not only diminish the pasture's carrying capacity but, it can wipe out perennial plant species, decrease nutrient cycling and accelerate soil erosion and an increase in low quality plant species or weeds and/or toxic plants and subsequently reduce animal performance.

Forages from marginal rangelands and crop residues are generally of low quality resulting in insufficient nutrient supply, low productivity and even bodyweight loss of animals when fed alone (Alemu et al. 2014). According to Buxton (1996), the nutritional values of forage species are low during the dry season compared to the wet season. The level of protein decreases rapidly with plant maturity; as the forages mature, they become dry and harder and nutrients become less accessible to animals. As a result of this decline in pasture quality, animals gradually lose weight as the dry season advances. Excessive loss of weight can impair the reproductive performance of an animal. As a general rule animals can lose up to 18% of their peak body weight without affecting the reproductive processes, but beyond this point fertility becomes severely affected (MAWRD, 1996).

Namibian rangelands have a wide of naturally occurring forage legume species and that have the potential to alleviate the feed scarcity and nutritional deficiencies, and at the same time reduce costs involved in purchasing commercial supplements. Forage legumes are high in CP and also have higher concentration of minerals, for example red clover and white clover are rich in protein and minerals such as calcium, but contain low levels of sugar and fibre (Dewhurst et al., 2009). Although forage Legumes are highly nutritious, some legumes species may contain considerable amount of anti-nutritional compounds for example tannins, alkaloids and saponins that may act as deterrents against feeding by herbivores, adversely affect animal performance or cause sickness or even death if consumed in large quantities (Alemu et al., 2014; Barahona et al.,

1997; Launchbaugh et al., 2001; Theodoridou, 2012). Furthermore, the high concentration of rapidly degraded protein in legumes also causes inefficient utilization of dietary nitrogen and increased urinary nitrogen output (Dewhurst et al., 2009). Also the high rates of release of soluble protein and break down into small particles from some forage legumes such as Lucerne and clovers is associated with bloat, primarily when consumed at an immature stage (Dewhurst et al., 2009; Thompson et al., 2000).

## **2.2 Goals of protein nutrition in ruminants**

The relative composition of feedstuffs varies widely in their proportions of protein and non-protein N (NPN), in the rate and extent of ruminal degradation of protein and in the intestinal digestibility and amino acid (AA) composition of ruminally undegraded feed protein (NRC, 2001). Ruminally synthesized microbial CP (MCP), ruminally undegraded feed CP (RUP) and to a very limited extent endogenous CP (ECP) contributes to the passage of metabolizable protein (MP) to the small intestine (NRC, 2001). By definition, the metabolizable protein is the true protein that is digested post-ruminally and the component AA absorbed by the intestine (NRC, 2001). The absorbed AA that serve as the building blocks for protein synthesis are vital to the maintenance, growth, reproduction and lactation. It is presumed that just like in monogastrics, an ideal pattern of absorbed AA exists for each physiological function in ruminants (NRC, 2001).

Ruminant protein feeding aims at providing adequate amounts of rumen-degradable protein (RDP) to optimize rumen function and to attain the desired productivity with a minimum amount of dietary CP. Optimizing use of dietary CP requires use of complementary feed proteins and NPN supplements that will meet requirements of rumen microbes for maximal synthesis of MCP; also the types and amounts of RUP should optimize the profile and amounts of absorbed

AA (NRC, 2001). There are indications that the nutritive value of MP for dairy cattle is determined by its profile of essential amino acids (EAA) and also by contribution of EAA to MP. Given the differences in forage legume composition, there is a possibility rations from forage legumes could be formulated that optimize productivity for a given physiological function, for example growth, which could be an aspect to investigate in future research.

### **2.3 Forage legumes as key role players in enhancing sustainability**

Legumes refer to shrubs, forbs and trees that are characterized by multi-leaf stalks (trifoliolate) that bear fruit in the form of a pod containing seeds. These plants may complete their life cycle within a single growing season (annuals); some may require two years to complete the life cycle (biennials); others may persist for many growing seasons (perennials). Forage refers to a plant or vegetative parts of a plant that is consumed or harvested and fed to domestic animals as fresh, dry or silage. Legumes belong to the plant family *Leguminosae* (Olalekan and Bosede, 2010) and are second only to grasses in importance to human and livestock nutrition (Graham and Vance, 2003). The well-known legume plants include beans, peas, peanuts, lentils, lupins, soybean, alfalfa, clovers and *Leucaena leucocephala* which are mostly used for seed production and/or herbage for livestock feed.

Nitrogen fixation refers to the capture of literally unlimited atmospheric nitrogen gas ( $N_2$ ) to form ammonia ( $NH_3$ ) which is readily available for assimilation by plant and microbes (Gresshoff et al., 2015). The primary role of legume species is in fixing atmospheric nitrogen into the soil and can lead to improved soil fertility, increase forage yield and enhance forage quality (Ledgard and Steele, 1992; Olalekan and Bosede, 2010) and increase nutritive value of pastureland by supplying nitrogen to the adjacent plants and as a result high quality animal feed can be produced. Most leguminous plants contain symbiotic nitrogen fixing bacteria within root

nodules of their root systems. Thus, legumes grown in soils poor in nitrogen can achieve dynamic growth without the nitrogen fertilizer that may be required by other plants. Their ability to fix atmospheric nitrogen into the soil make them excellent components within different farming systems because they provide residual nitrogen to non-legume plants which minimises the need for mineral nitrogen fertilizers by non-legumes.

The amount of atmospheric nitrogen fixed by legumes varies between species due to the relative effectiveness of the symbiosis between the plant and the bacteria, soil and environmental conditions and for this reason their effect on soil fertility is also likely to be variable under different management regimes (FAO/IAEA, 2000). Perennial legumes predominantly derive their N from N<sub>2</sub> fixation, especially when intercropped with grasses. As such, a good correlation exists between N<sub>2</sub> fixation per ha and year and legume dry matter yield, suggesting that management factors that favour legume productivity should result in high N<sub>2</sub> fixation (Carlsson and Huss-Danell, 2003). For example, frequent defoliation in spring increased N<sub>2</sub> fixation in *Trifolium repens* pastures (Ledgard et al., 1992).

Apart from the ability to fix atmospheric nitrogen into the soil, legumes contain significant amount of nutrients that are lacking in other feed resources. They are typically known to have higher protein content than grass species (Adesogan et al., 2002; Paulson et al., 2008). Leguminous species have high CP content, ranging from 10 – 25% on dry matter basis (Onyeonagu and Eze, 2013; Okoli et al., 2003) and the nutritive values of legume species declines only slowly with maturity (Norton, 1982). Legume fibre tends to digest faster than that of grasses allowing ruminants to eat more of the legumes (Onyeonagu and Eze, 2013). Legumes species can also supply significant amounts of energy, vitamins and minerals in addition to protein (Olalekan and Bosede, 2010) and animals tend to consume legumes at higher rate than

grass species (Baumont, 1996). Grass species are generally moderate to low in crude protein and contain more Neutral Detergent Fibre (NDF) as compared to legumes (Alemu et al., 2014).

In order to improve livestock production, there is a need to improve the quality and quantity of available forage through feed supplementation. Leguminous trees and shrubs are important components of ruminant diets especially during the dry season, when most other feed resources depreciate in quality and quantity (Jamala et al., 2013). Legumes have a slower rate of decline in digestibility with advancing maturity compared to grasses. This has been shown in red clover (Thomas et al. 1981) and white clover (Ulyatt, 1970), for instance.

Supplementing with forage legumes can enhance the utilization of poor quality roughages in smallholder mixed farming systems. High protein containing legumes, in particular shrub species, can contribute to improved productivity of livestock during the dry season and at the same time maintain and even restore soil fertility (Andersson, 2006). The importance of browse increases with increasing aridity and is generally most essential in the dry seasons, when most other feed resources devalue in quality and quantity (Khan et al., 2003) and Namibia is no exception.

Forage legumes used either as silage or grazed herbage lead to higher intake and animal production than grass silages of comparable digestibility (Dewhurst et al., 2009). Supplementing poor quality basal grass diets with legume forage increased feed intake and diet digestibility by ruminant livestock which in turn enhance livestock performance (Foster et al., 2009; Alemu et al., 2014). Supplementation of sheep in Zimbabwe with forage legumes improved nitrogen retention and reduced weight losses (Baloyi et al., 2008).

Introduction of forage legumes in rangelands also improves the ecosystem through nutrient cycling, litter fall, biomass input and biological nitrogen fixation (Gathumbi et al.,



2003). While legumes provide the ground cover for bare soil as high mulching value for erosion control, they can contribute to the improvement of quality of standing hay, maintain and restore soil fertility largely due to their ability to fix atmospheric nitrogen (Olalekan and Bosede, 2010). Moreover, legume species remain evergreen for a longer period in many marginal rangelands (Oji and Kalio, 2004). They have the ability to extract water and nutrient from the deep water table throughout the year (Aregawi et al., 2008; Abraham et al., 2016) and continue to produce well into dry season (Paterson et al., 1998). This study therefore sought to identify native forage legumes in the rangelands of central Namibia and the possible role they could play in improving nutrition of ruminants.

There are differences among legumes in their potential for nitrogen fixation and soil N uptake. Research in Australia has shown benefits to production and N cycling with perennial legumes as opposed to annual legumes in dry-land pastures (Ledgard, 2001). Studies with perennial pastures containing Lucerne (*Medicago sativa* L.) showed consistently greater production, more stable legume content and 90 – 150% more N<sub>2</sub> fixation than annual pastures with subterranean clovers (Peoples et al., 1998). Species selection in perennial pastures can also influence productivity and efficiency of N cycling. Ruz Jerez (1991) reported in a sheep-grazing study that a mixed herbal pasture containing 11 grasses, 7 legumes and 5 herbs produced one-third more dry matter than a white clover/perennial rye grass pasture and was similar to that for ryegrass-alone fertilized with 400 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The better growth of the mixed pasture largely occurred during summer by the deep-rooted clover (*Trifolium pretense* L.) and chicory (*Cichorium intybus* L.). The mixed pasture had higher N<sub>2</sub> fixation; the mixed pasture also had higher annual N uptake (42%) than the clover/grass pasture and may reflect greater N recycling in the excreta combined with greater recovery of excreta-N by the deep-rooted species. The high

N uptake by the mixed herbal pasture may have been due to increased mineralisation of N in soil (Ruz Jerez, 1991). This underscores the need to evaluate the performance of consortiums of legumes and how they could be optimized through grazing management.

#### **2.4 Roles of forage legumes in improving protein utilization in ruminants**

The protein contained in forages provides both ruminal degraded protein (RDP) for microbial protein synthesis and ruminal undegraded protein (RUP) that escapes microbial breakdown (Broderick, 1995b). In general, rapid and extensive degradation of protein in legume and grass forages in the rumen results into decreased protein efficiency. Forage N occurs as both protein and nonprotein N (NPN). Constituents of forage NPN include oligopeptides, free amino acids, ammonium compounds and other small molecules that rapidly contribute to the ruminal ammonium pool (Broderick, 1995b). When there is an energy deficit but with an excess of peptides and amino acids of plant origin, rumen microbes use amino acids for energy and liberate ammonia through deamination (Kingston-Smith and Theodorou, 2000). With abundant fermentable carbohydrates in the rumen, more non-protein N and amino acids can be taken up by rumen microbes and incorporated into microbial protein (Simili da Silva et al., 2014).

The properties of a protein in forage determine the proportion of its CP that is RDP and RUP. If degradation of protein is fast, all the amino acids and ammonia released cannot be utilized efficiently and less protein is synthesized than is degraded (Broderick, 1995b). The quantity of microbial protein formed in the rumen is related to dietary digestible energy (DE) rather than protein (Broderick and Merchen, 1992). Forages high in water soluble carbohydrates (WSC) may improve balance and synchrony of the carbon (C) and nitrogen (N) (Miller et al., 2001) and increase microbial protein production in the rumen and animal productivity (Parsons et al., 2011). When nonstructural carbohydrates (NSC) were increased in alfalfa, there was a

significant reduction in ruminal pH (6.85 vs. 7.08) and NH<sub>3</sub>-N concentration in an *in vitro* continuous culture system (Berthiaume et al., 2010), increased *in vivo* protein synthesis by rumen bacteria (Brito et al., 2009) and improved milk yield of dairy cows in late lactation (Brito et al., 2008). With more fermentable sugars available in the rumen, it has been reported there is a reduction in rumen ammonia (Lee et al., 2002; Hristov et al., 2005). On forage silage diets it is apparent that provision of sufficient fermentable energy (e.g. from ground high moisture corn) is critical for stimulating microbial capture of RDP. When animals RDP intake exceeds microbial utilization, it contributes to preduodenal N losses, increases energy requirements of animals (Cannas et al., 2004) and reduces embryo survival in sheep (Berardinelli et al., 2001; Fahey et al., 2001).

Evidence suggests that protein is the first nutrient that limits milk production when high quality silage is fed to dairy cows (Dhiman et al., 1993). In the study of Dhiman et al. (1993) they obtained greater response to RUP than to energy, which supports the hypothesis that absorbed protein (AP) supply to the intestine is inadequate in dairy cows fed large quantities of high-protein legume silage. In a comparison of raw soybeans, roasted soybeans and solvent soybean meal (SBM), Faldet and Satter (1991) found that that the roasted soybean diet although containing the same amount of energy as in raw soybeans, supported 4.7 kg/day greater milk yield. Milk yield was similar for solvent SBM and raw soybeans, which indicated that the higher yields from roasted soybeans was due to RUP and not energy. Muck (1991) indicated that controlled heat treatment of alfalfa hay was a possible means of protecting the protein from ruminal breakdown.

In an experiment with yearling heifers that compared alfalfa ensiled at 44% DM (high moisture) or 64% DM (low moisture) to dehydrated alfalfa by measuring their effect on plasma

concentrations of branched-chain amino acids (BCAA), there was an increase in BCAA of 74, 227 and 298 nmol/mL with feeding of high moisture silage, low-moisture silage and dehydrated alfalfa, respectively (Roffler and Satter, 1985). Amino acid uptake from the intestine is indicated by the plasma BCAA concentration (Bergen, 1979), hence the greater elevation of BCAA in hydrated alfalfa (which is heated during processing) and low-moisture silage (which undergoes more heating and less proteolysis) than high moisture silage, supplied more RUP and more amino acids for absorption in the intestine. Feeding steam-heated hay (17% CP) as a replacement of unheated hay or alfalfa silage at approximately 25% of dietary DM, increased DMI and yield of milk and milk components (Broderick et al., 1993b). Cows fed the heated hay diet had comparable milk yield to those fed a solvent SBM (23% CP). It should be noted, however, that steam-heating alfalfa reduced rumen protein degradability, although energy digestibility was also reduced (Broderick et al., 1993b).

Broderick (1995b) compared the performance of lactating cows fed all their forage as either alfalfa silage (AS) or alfalfa hay (AH). The protein adequacy of the diets was assessed based on the cows' response to supplementation with fish meal, which is a high-RUP protein source. Fish meal increased mean protein yield in both the silage and hay diets, but the response was greater in alfalfa hay diet. Although the AS and AH had similar RUP content (V.D. Peltekova, 1994, unpublished data as cited by Broderick 1995b), microbial protein synthesis was 29% greater in AH. It was suggested that the release of total amino acids and ammonia in AH may have been more synchronous with ruminal energy fermentation thus supporting greater microbial capture of RDP by rumen microbes on AH than on AS diets (Broderick, 1995b). Reduction of proteolysis and NPN formation in a silo can improve forage protein utilization in lactating dairy cows (Broderick, 1995b). Steam treatment, use of formic acid or formaldehyde on

alfalfa before ensiling all reduced proteolysis in the silo by reducing silage NPN (Charmley and Veira, 1990).

The nutritional value of forage legumes for feeding ruminants can be improved by: (1) reducing the rate and extent of ruminal protein degradability; (2) reduction of proteolysis and NPN formation in the silo; and (3) increased microbial protein formation in the rumen (Broderick, 1995). Rates of proteolysis after ensiling differ among forage species. Silage from red clover (which does not contain tannins) contains much less NPN than alfalfa (Papadopoulus and McKersie, 1983). Mixing red clover and alfalfa extracts led to substantial reduction in proteolysis and this is explained by the presence in red clover of a polyphenol oxidase system that produces phenolic compounds that inhibit proteolysis during ensiling (Jones et al., 1995). The implication is that silages from different forage legumes may have different nutritive value and this may be different from grazed fresh material; blending legumes may be beneficial during ensiling (Broderick, 1995b).

Silage from legumes containing condensed tannins (e.g. *Sericea lespedeza* and *Lotus pedunculatus*) and the non-tannin legume, red clover all had lower NPN proportion than alfalfa silage (Albert and Muck, 1991). Studies using several temperate adapted forage legumes (including crown vetch, *Sericea lespedeza*, *Lotus pedunculatus*, alfalfa and birdsfoottrefoil) have shown that reduced degradation rate and increased ruminal escape were proportional to tannin concentration (Broderick and Albrecht, 1994). Tannin concentration, however, only offers a partial explanation in the differences observed in ruminal protein degradation among forage legumes, as is evident with the polyphenol oxidase system in red clover discussed above (Broderick, 1995).

The efficiency with which soluble N is utilized in the rumen will depend on the rate of release and the concentration of carbohydrates and N availability (Hristov et al., 2005). High energy availability combined with reduced total N concentration or N solubility can optimize microbial synthesis and improve N utilization (Bryant et al., 2012). Efficiency of utilization of forage proteins in dairy cows and other ruminants can be improved in several ways. Ration formulation may be used to feed more RUP or to rectify excessive degradation of forage proteins. Maximizing fermentable energy in the ration will maximize capture of degraded forage protein for protein synthesis in the rumen, provided cows are fed a minimal amount of effective fiber (approximately 28% NDF on DM basis) (Broderick, 1995b). This can be achieved by feeding higher concentrate levels, more finely grinding the concentrate part of the ration and substitution of part of the forage for example alfalfa silage, with higher-energy forage like corn silage. Red clover silage (with low NPN content) may be used to partially substitute high-NPN silages made from alfalfa or other legumes (Broderick, 1995b). Alfalfa hay has more slowly degraded protein which may result in more efficient utilization of its RDP for microbial protein synthesis. Forage legume hays may in general be more used efficiently on account of their RDP being more slowly degraded in the rumen compared to silages (Broderick, 1995b).

Parsons et al. (2011) reported that improvements in the forage WSC/CP ratio led to concomitant increases in N use efficiency in dairy cows. In a study done with late-lactating dairy cows, increasing the WSC/CP ratio of alfalfa baleage from 0.49 to 0.62 with PM- vs. AM-cutting increased protein synthesis and  $\text{NH}_3\text{-N}$  uptake by rumen bacteria, implying an improvement in N utilization (Brito et al., 2009). Different pasture legume-grass configurations will vary in the WSC/CP and therefore can affect the N utilization efficiency (Simili da Silva et al., 2014). Species selection in legume-grass pastures can result in significant variations in the readily

available energy to protein ratios and of carbohydrate and protein fractions together with variations in DM yield and other nutritive characteristics (Simili da Silva et al., 2014). This emphasizes the importance of correct choice of legumes to combine with grasses in pastures for different agro-ecological zones.

In their review, Edwards et al. (2007) and Parsons et al. (2011) concluded that the response of N use efficiency in dairy cows to forage WSC/CP ratio was continuous and hence any improvements in the WSC/CP ratio would be of benefit in use of dietary N for milk production and reduction of N losses in urine. Another strategy to improve readily-available energy to protein ratio of forages is harvesting forages in the afternoon (Morin et al., 2011, 2012), this is due to increased WSC levels because of accumulation of photosynthetic products (Holmes et al., 2007).

## **2.5 Desirable characteristics of forage legumes used in animal supplementation**

Pastures in some parts of the world commonly include grass-legume mixtures due to the nitrogen fixing capability of legumes, whereby some of the nitrogen gets eventually transferred to the associated grasses (Hill et al., 2009). While grasses have considerable seasonal variation in feeding value, legumes provide forage of consistently high digestibility and protein content.

Due to the extensive nature of beef and small tock farming in Namibia or competition for land to grow crops, only a small portion of the total may be available for fodder production, hence the role of legumes will be to increase the efficiency of utilization of the basal diet at low levels of supplementation (less than 20%). The legume should have a high protein content to supply both fermentable and bypass protein. Additional benefits arise if the legume contains other critical nutrients (for example lipids, minerals, vitamins and other plant compounds) which enhance the rumen ecosystem, leading to increased microbial growth, rate of fibre digestion,

propionate production and escape of dietary protein (e.g. tannins) (Preston, 1980). Legumes high in tannins may be superior as sources of bypass protein, since tannins link with proteins during mastication and appear to reduce microbial degradation of plant proteins (Reid et al., 1974).

Although *Letus pedunculatus* is high in tannins which protect protein from degradation, they reduce fibre digestibility by inhibiting bacteria (Chesson et al., 1982) and fungal activity (Akin and Rigsby, 1985). The ideal condensed tannin (CT) concentration in forages is 20-40 g/kg diet DM; higher levels (70-90 g/kg DM) are detrimental (Barry, 1985; Waghorn et al., 1994). *Leucaena*, *Gliricidia* and *Sesbania* are examples of tropical forages containing tannins. Forages containing high tannin concentration may be less palatable than those with low or no tannins, however, animal performance may be better on forages with high tannin level. Trefoil and sainfoin which are tannin containing legumes, were less palatable than lucerne, but supported faster growth rate in heifers and this was attributed to more escape protein due to the tannins (Marten and Ehle, 1984).

At concentrations of 6% or more, condensed tannins reduce forage quality (Waghorn et al., 1994) but concentration of 2-4% of DM usually result in improved forage nutrient utilization by ruminants (Min et al., 2003, 2005). According to Waghorn et al. (1994) consumption of *L. pedunculatus* which has high CT contents (> 5% DM) may adversely affect feed intake; medium or low CT contents (< 5% DM) appear to have negligible effect on intake.

## **2.6 Legume importance in grazing systems**

Legumes play an important role in temperate regions in improving the feeding value of dairy cattle (Ulyatt, 1973; Stiles et al., 1968), beef cattle (Yarrow and Penning, 2001) and sheep (Fraser et al., 2004) pastures and also provide low-cost N from biological nitrogen fixation. Increasingly they are being viewed as an important component of environmentally sustainable



grassland ecosystems due to their: persistence under grazing; ability in improving soil structure; and potential of legumes with condensed tannins to reduce methane emissions (Waghorn et al., 1998; Waghorn et al., 2002).

A case in point to illustrate the importance of forage legumes is New Zealand. The main competitive advantage of New Zealand's dairy industry is the low prices of milk which are attributed to utilization of grass/legume pastures (Woodfield and Clark, 2009). White clover is the main legume incorporated in pastures. Although intensification led to a large increase in the use of nitrogen fertilizer in the period 1988 to 2008 in the New Zealand dairy industry, the high cost of nitrogen fertilizer relative to milk prices, requires farmers to re-assess the long-term role of legumes in pastures and how management could be changed to promote higher clover content under intensive grazing (Woodfield and Clark, 2009). In the EU, intensification of livestock farming has been accompanied by the development of maize silage and intensively fertilized grasses at the expense of forage legumes. Nonetheless, there is renewed interest in forage legumes because they only require solar energy for N<sub>2</sub> fixation, hence reducing energy consumption and associated impacts. Forage legumes contribute to reducing the global warming potential of livestock systems by reducing emission of enteric methane and nitrous oxide from pasture and crop production (Peyraud et al., 2009).

The higher intakes observed with forage legumes is pivotal to higher performance, since effects on feed efficiency are inconsistent (Dewhurst et al., 2009). Dairy cattle have increased DMI when on legume-grass pastures relative to grasses and this is attributed to lower NDF and higher digestibility of legumes compared to grasses (Kristensen et al., 2005). Dairy ewes grazing legume-grass pastures had higher pasture protein intake and DMI (Molle et al., 2008). White clover plays an important role in grazing systems in New Zealand because of its higher

nutritive value, high intake characteristics and faster rates of passage which contribute to higher milk production than for perennial rye grass (Thomson et al., 1985; Johnson and Thomson, 1996). In New Zealand, the white clover content of pastures averages 10-20% (Woodfield and Clark, 2009), but Harris et al. (1997) demonstrated that maximum milk production was achieved with white clover content in excess of 50%. Grazing trials have also demonstrated that other legumes like birdsfoot trefoil (*Lotus corniculatus*) can give higher milk production than white clover dominant swards (Harris et al., 1998), but unfortunately birdsfoot trefoil has lower herbage yield and poorer persistence under intensive grazing management than white clover (Woodfield and Clark, 2009).

Red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) are examples of other perennial legumes that are used to varying degrees in dairy farming systems. In high fertility pastures under low intensity grazing, red clover performs well but fails to persist beyond 3 years under intensive grazing (Cosgrove and Brougham, 1985). High herbage yields can be achieved with lucerne, but difficulties with grazing it *in situ* during winter and early spring have limited its use in current grazing systems in New Zealand (Woodfield and Clark, 2009). Presently the main use of lucerne is as a conserved forage, although breeding research is underway to make it more tolerant to grazing (Woodfield and Clark, 2009).

The perennial ryegrass/white clover pastures which are not irrigated form the base for New Zealand's intensively managed pastures with a maximum dry matter yield of 15 – 16 t ha<sup>-1</sup> year<sup>-1</sup> (Clark et al., 2001). This system has been successful due to high stocking rates and seasonal calving which ensures that pasture supply matches animal demand (Woodfield and Clark, 2009).

As grasses are actively growing in spring, the frequency and intensity of defoliation become critical factors in persistence of white clover plants. Application of phosphate and sulphate fertilizers stimulates white clover growth and nitrogen fixation and subsequently grass growth (Woodfield and Clark, 2009). A promising approach that does not use grass/legume mixtures, is growing clover and grass as adjacent monocultures which increased milk yield by 2 to 3 kg cow<sup>-1</sup> day<sup>-1</sup> in early lactation (Nuthall et al., 2000). Dairy sheep grazing grass-legume spatially adjacent monocultures (SAM) had increased milk production compared with grass legume mixtures (Champion et al., 2004; Molle et al., 2007). Sheep grazing perennial ryegrass-white clover SAM, predominantly (62%) selected white clover; the selected proportion of legume is indeed close to the 3:7 ratio of grass to legume that was reported to optimize rumen microbial activity based on in vitro experiments (Merry et al., 2002).

Use of lower stocking rates (e.g. 2.2 cows/ha under New Zealand conditions) may provide farmers with the option of including legumes in their pastures without reducing milk yield per hectare. Higher legume content in pastures could be achieved through decrease in fertilizer N use, consideration of alternative grass species, better forage conservation and having larger areas of pasture renewal (Woodfield and Clark, 2009). To improve the persistence of white clover in New Zealand, selection for increased stolon density is important (Woodfield et al., 2001).

Wild relatives of currently used forage legumes like white clover in New Zealand, despite having poor agronomic value are being explored (Williams et al., 2007) as sources of genes for useful traits such as drought tolerance, salinity tolerance, pest and disease resistance, establishment vigour and forage quality (Woodfield and Clark, 2009). This research examined

the potential role native legumes could play in fodder production for feeding of ruminant livestock, by determining the nutritive value of fourteen (14) legumes found in central Namibia.

## **2.7 Legume utilization strategies**

Legumes can be planted in combination with grasses forming mixed grass-legume pastures, for example white clover/ryegrass pastures in New Zealand and France (Peyraud et al., 2009; Woodfield and Clark, 2009). Due to its poor persistence red clover is mainly used for silage production (Le Gall, 1993). Lucerne is predominantly used for silage and hay making, but is also used as dehydrated forage in France (Peyraud et al., 2009). Controlled grazing of natural pastures improved with a legume (“protein bank”) can also increase productivity. For example, the inclusion in natural pasture of the forage legume *Lotus uliginosus* which was reported to supply 43 – 62% of its N as un-degradable protein (Quirama et al., 2011) was effective in attaining target growth rates of weaned lambs (Piaggio et al., 2015). Spatially adjustment mixtures (SAM) have also been investigated as an alternative way of supplying supplemental protein (Champion et al., 2004; Molle et al., 2007).

The wide variation seen among legumes in plant morphology, chemical composition, growth habits suggests it may be beneficial establishing multi-species legume pastures in rangeland rehabilitation (Muir et al., 2001). Although legumes can fix nitrogen, there is wide variation among individual species in N fixation (Hiers et al., 2003; Muir et al., 2005b). Wide variation among warm season legumes native to southern north America have also been observed for growth form, adaptability, competitiveness and acceptability to grazing herbivores (Diggs et al., 1999; Muir et al., 2005b).

## 2.8 Protein supplementation effects in ruminants

Protein supplementation is common in many production systems where cattle are fed low-quality forages because this can improve forage use and performance (McCollum and Horn, 1990). The CP content of a basal diet can be used as a rough indicator of the potential of livestock to respond to supplemental protein (McCollum and Horn, 1990; Van Soest, 1994; Moore and Kunkle, 1995). A threshold of response to supplementation in the basal diet is 7% CP, such that when CP is greater than 7% there seems to be little benefit to supplementation (Mathis et al., 2000).

Effects of supplementation with RDP on cattle fed low- to medium quality forages have been variable, when the CP content in the basal diet is below the 7% threshold (Heldt, 1998). There is a diminishing response to increasing RDP, which indicates the potential to stimulate intake via RDP has limits. The characteristics of the forage being fed (e.g. inherent fermentability and protein availability) and the animal's nutrient status (e.g. relative protein demand) will set limits to how responsive intake will be to provision of RDP (Koster et al., 1996). Increased forage intake in response to protein supplementation tends to increase rates of passage, ruminal fill or both (McCollum and Horn, 1990). In some instances, improved digestion may contribute to intake and passage responses (McCollum and Horn, 1990).

Supplementation of low-quality forages (< 6% CP) with CP increases DM, OM and NDF digestibility (DelCurto et al., 1990; Horney et al., 1996; Bohnert et al., 2002b). This is due to increased N availability to the ruminal microflora which increases ruminal fiber digestion (Campling et al., 1962; Petersen, 1987). Crude protein supplementation of beef cows consuming low-quality forages has routinely improved body weight and BCS (body condition scores)

change compared to not supplementing with CP (Horney et al. 1996; Bohnert et al., 2002; Currier et al., 2004a).

There is a positive correlation between digestibility of feeds and intake in ruminants (McDonald et al., 2010). Adult sheep feeding barley straw and supplemented with 150, 300 and 450 g/d of Lucerne hay had linear increases in dry matter digestibility (DMD) and organic matter digestibility (OMD) (Haddad, 2000). Linear increases in DMD and OMD were reported when urea treated barley straw was supplemented with 25 or 36% Lucerne hay, although no further increases in digestibility occurred when Lucerne hay was included at 45% of the diet (Abate and Melaku, 2009). Similarly digestibility increased with increased Lucerne silage supplementation in steers (Zhuoga et al., 2016).

It has been suggested that supplementation with protein or energy improved forage DMI (dry matter intake) when forage TDN to CP ratio exceeded 7, indicating N deficiency (Moore et al., 1999). The ratio approach, has in general, proved to be most beneficial to cattle consuming low quality forages in which DIP (degradable intake protein) is limiting (Hersom, 2008). This study investigated the effect of different forage legume supplementation regimes of weaned Boer goats feeding on basal grass hay on voluntary feed intake and body weights.

## **2.9 Nutritive value of forages**

The Nutritive value of forage is a function of feed intake and the efficiency of extraction of nutrients from the feed during digestion (Gutteridge and Shelton, 1998; Mandal, 1997). Saha et al. (2013) define nutritive value as a feed's protein, mineral and energy composition, availability of energy, and efficiency of energy utilization. The nutritive value is determined by its ability to provide the nutrients required by an animal for growth, reproduction, lactation, production and maintenance. The feeding value of pasture can be altered by changes in species

composition of the sward. Generally, higher milk yields are obtained with swards of pure pastures than grasses (Holmes et al., 2007). The nutritive value of a feedstuff can be assessed by analysing its chemical composition followed by measuring palatability and digestibility.

## **2.10 Chemical constituents of feed**

### **2.10.1 Crude Protein (CP)**

Proteins and energy are the most important nutrients for livestock because they support rumen microbes that consequently degrade forage. According to Newman et al. (2009), forage protein concentration differs significantly depending on plant species (legume/cereal/grass), soil fertility and plant maturity. Legumes contain higher CP compared to other species e.g. CP concentration of alfalfa ranged between 18–25%; in corn leaves it ranged between 6–14%; while CP concentration for Coastal Bermuda grass leaves ranged between 4–18%. Crude protein is measured indirectly from the N content of the feed sample and multiplying by 6.25, the average nitrogen content of protein (NRC, 2001).

### **2.10.2 Neutral detergent fibre (NDF)**

NDF can be defined as the residual or insoluble fraction which is left after boiling the feed sample in neutral detergent solution during laboratory analysis. Neutral detergent fibre measures most of the structural components of plant cells (i.e. cellulose, hemicelluloses and lignin) (NRC, 2001). Unlike lignin, hemicellulose and cellulose can to a certain extent be digested by microorganisms in the rumen of ruminants or hind-gut of monogastric animals (Saha et al., 2013). The levels of NDF in forage tend to increase with maturity. NDF concentration is negatively correlated with dry matter intake. For instance, as the level of NDF in the forage increases, voluntary feed intake tends to decline (Robinson et al., 1998), hence NDF is often used in formulas to predict the dry matter intake by livestock. A decrease in dietary NDF

concentration is correlated with greater particle size reduction through mastication and fermentation (Meissner and Paulsmeier, 1995; Ellis et al., 2005).

The extent to which cellulose and hemicelluloses are fermented varies with the lignin content of the cell wall (Van Soest, 1967). Lignin's effects may be mediated through the cross linkages it forms with the structural carbohydrates of the cell wall (Van Soest, 1977). The extent of cell wall degradation is dependent on the digestion rate of the cell wall, the retention time (the time taken for the digestion rate to operate on this wall) and the amount of potentially digestible cell wall in the forage (Demment and Van Soest, 1985).

Neutral detergent fibre (NDF) has been suggested for use in defining the upper and lower bounds of DMI (Mertens, 1994). When the NDF concentration in the diet is high, DMI intake is restricted by rumen fill; on the other hand at low NDF concentration, DMI is limited by energy intake feedback inhibitors. In cattle, DMI generally declines when diets exceed 25% NDF. At a given NDF concentration, however, there is a considerable range in DMI among NDF sources in the diet which may be attributed to particle size, digestibility and rates of passage from the reticulorumen (Allen, 2000).

Digesta passage from the rumen is inversely related to the extent of digestion within the rumen (Kammes and Allen, 2012). During digesta passage, there is selective retention of undigested fibre, which permits ruminants to increase ruminal fibre digestion (Allen and Mertens, 1988), but extended ruminal retention times of the retained fibre can decrease DMI from ruminal distention (Allen, 1996). Passage from the rumen is influenced by particle size, although this is not always a constraint because a large proportion of the particles retained in the rumen are smaller than the maximum particle size in the faeces (Allen, 1996). Other factors that influence passage of particles from the rumen are particle density and buoyancy (Jung and Allen,



1995) and sequestration of small particles within the fibrous rumen mat (Sutherland, 1988). The rate of passage of fibrous particles depends on the reduction of particle size and the increase in particle specific gravity to permit particles to escape the rumen mat, sink to the ventral rumen and exit the rumen via the reticular-omasal orifice (Sutherland, 1988).

In a study that involved feeding dairy cows with alfalfa or orchard grass as the sole source of forage, the alfalfa diet significantly increased the rate of particle size reduction of indigestible Neutral detergent fibre (iNDF) from large to small compared with orchard grass (Kammes and Allen, 2012). This was attributed to the greater resistance of grass cell walls to particle breakdown than alfalfa cell walls because of chemical and structural differences (Wilson and Hatfield, 1997). The faster particle size reduction rate indicated that alfalfa NDF was more fragile than orchard grass NDF (Kammes and Allen, 2012). A greater pool of large fibrous particles for orchard grass is believed to have entrapped small particles preventing their sedimentation, hence the probability of escape. Besides particle size, shape of particles within the rumen mat may be important. Fragments of legumes are cuboidal-shaped, usually passing faster than grass particles which are elongated and needle like (Buxton et al., 1996). Because of the intertwining of the thin grasses particles within the rumen mat, they may be more efficient at retaining small particle than those of legumes (Kammes and Allen, 2012). Selective retention of small particles was less for alfalfa than orchard grass resulting in lower rumen fill and less effective fibre (Kammes and Allen, 2012).

The digestibility of NDF is an important determinant of the energy value of a given forage. For instance Tifton 85 Bermuda grass has higher digestibility relative to other varieties of Bermuda grass despite a similar or higher NDF content, which should be considered during ration formulation (West et al., 1999). In forage-based diets, nutrient supply and utilization are

affected by maturity of the forage at harvest. Although delayed harvesting of pasture generally increases DM yield, nutritive quality decreases as NDF concentration increases and the digestibility of the pasture decreases due to greater lignification (Kammes et al., 2012).

An optimum density of fibre in their food may be required by herbivores to access cellular contents, as fibre provides a rigid material against which cells are pressed until its rupture during chewing (Wright and Vicent, 1996). Additionally, a high fibre intake rate is correlated to higher volume of salivary buffer produced to achieve a greater ruminal pH in ruminants fed diets rich in tannins (Makkar, 2010). This would enhance fibre digestion, resulting in more energy available to the animal (Weimer, 1996).

### **2.10.3 Acid Detergent Fibre (ADF)**

ADF refers to the fibre fraction or the residue that remains after boiling a forage sample in acid detergent solution. It is the constituent which represents the least digestible fibre fraction of feedstuff; the portion that does not dissolve in acid detergent solution. ADF is a sub-fraction of NDF which consist of lignin, cell wall components of cellulose, silica and insoluble forms of nitrogen but not hemicellulose (Saha et al., 2013). Lignin and silica are indigestible whereas cellulose can be digested by the rumen microbes (Omoniyi et al., 2013) therefore, low ADF values are desirable.

ADF is useful in predicting digestibility and energy content in of a forage. ADF is negatively correlated with forage digestibility. Forages with higher ADF are lower in digestible energy than forages with lower ADF (Omoniyi et al., 2013); hence when ADF level in a forage increases, the digestibility and energy value of such forage decreases. Forage crops increase in

ADF as they mature. According to Newman et al., (2009), ADF is often used to calculate digestibility, total digestible nutrients (TDN) and/or net energy for lactation (NE<sub>L</sub>).

In a study where cows were offered diets containing 14, 17 and 20% ADF, they consumed more feed when offered the diets with lower fiber during cool and hot weather (Cummins, 1992). All treatment groups showed a decline in intake as the ambient temperature increased, but the decline was most rapid for cows receiving low fiber diets compared to the ones offered the high fiber diets. This suggests that total energy intake and the resulting metabolic heat production may have a greater influence on DMI than fiber content of the diet (West et al., 1999).

#### **2.10.4 Ash**

Ash is the inorganic residue that remains after water and organic matter have been removed by heating in the presence of oxidizing agents. The ash content is the total amount of inorganic matters such as minerals (Calcium, Potassium, Phosphorus etc.). Unlike other feed components, minerals have a low volatility, thus they are not destroyed by heat. The three main types of analytical procedure used to determine the ash content of foods are dry ashing, wet ashing and low temperature plasma dry ashing (McClements, 2003; Galyean, 2010).

#### **2.10.5 Ether extract (EE)**

Total fat or lipids are organic compounds that are relatively insoluble in water but, soluble in organic solvents such as ether, chloroform and benzene (Romero et al., 2014). Fats in feed are normally determined through ether extracts. According to Saha et al. (2013), EE is a portion of dry matter which is extracted with ether to estimate the total fat content of feed and it may include lipids of no nutritive value such as waxes, pigments and other lipids to a less degree

in addition to true fats, hence measurement of fat through EE is expressed as crude fat. Fatty acids, triglycerides and phospholipids are the significant fats in animal nutrition and constitute 1 to 3% of forage dry matter (Hatfield et al., 2007).

Fats are high density sources of energy for animals and provide essential lipid nutrients and are highly digestible; they typically contain 2.25 – 2.8 times more energy than either carbohydrates or proteins (Romero et al 2014; Saha et al., 2013), hence the higher the level of fat in feed the higher the metabolisable energy. Fats in feed can contribute to the overall physical characteristics e.g. appearance, flavour, texture and mouth-feel.

#### **2.10.6 Crude Fiber (CF)**

Crude fibre is defined (CF) as those variable amounts of lignin in feed which are not digestible (Saha et al., 2013). Crude fiber accounts for most cellulose and small portions of lignin but not ash; it underestimates the true fiber and is less than acid detergent fiber (ADF) (Saha et al., 2013). CF is not a very useful parameter for quantifying forage fiber where lignin content is substantial, nevertheless, it provides a reasonable estimate of the fiber in grains because of their low lignin content (Saha et al., 2013).

#### **2.11 Plant secondary metabolites (PSM)**

Plant secondary metabolites (PSM) or phytochemicals are a diverse group of natural products (Harbone, 2001; Wink, 2004), some of which may have nutritional value, but many of which have no nutritional value or antinutritional properties (Acamovic and Brooker, 2005). The PSM that have been most often identified are simple molecules for example alkaloids and amino acids, however, other more structurally complex compounds with physiological effects in animals have been found. These include proteins, peptides, carbohydrates and polyphenols (e.g.

tannins) (Acamovic and Brooker, 2005). Examples of PSM are mimosine (*Leucaena* species), saponins (Lucerne).

The primary reason for the existence of PSM is for plant defense (Acamovic and Brooker, 2005). Among the proposed functions are: defense against grazing herbivores and insects; defense against micro-organisms including bacteria, fungi and viruses; defense against other plants competing for nutrients and light; as a protection mechanism against the damaging effects of UV light (Acamovic and Brooker, 2005). Plant metabolites such as tannins deter herbivores from feeding on woody plant tissues possibly because they interfere with digestive or metabolic processes (Provenza et al., 1990).

The detrimental effect of secondary plant compounds becomes more evident when that foliage is the only feed consumed (Gutteridge and Shelton, 1998). PSM also play other roles including: acting as volatile attractants to promote pollination by birds and insects; colouring for the purpose of camouflage or attraction; signals to promote colonization by beneficial symbiotic micro-organisms for example mycorrhizal fungi and N –fixing rhizobia. A nutritional role during seed germination has also been suggested particularly for the N-containing PSM (Acamovic and Brooker, 2005). In the next subsections, we briefly review the PSM found in leguminous plants that are of importance in grazing ruminants.

### **2.11.1 Tannins**

Proanthocyanidins (PA) comprise flavan-3-ol (catechin) units condensed into polymers through C-C bonding and are synonymous with the term ‘condensed tannins’ (Waghorn and McNabb, 2003). Leaves of browse plants and seed coats commonly contain PA; leaves of temperate legumes usually contain lower concentrations of PA. Grasses have very low concentrations of PA or none at all (Waghorn and McNabb, 2003).

Tannins are secondary compounds that naturally occur mainly in woody species and are widely spread among forbs, shrubs and trees (Launchbaugh, 2001; Acamovic and Brooker, 2005). Tannins occur commonly in both woody (about 80%) and herbaceous (about 15%) dicotyledonous plant species (Provenza et al., 1990). Ingested tannins have both negative and positive effects on feed intake, digestion and animal performance. They have the ability to form insoluble complexes with dietary proteins in the rumen which reduces the digestion of forage protein, hence the amount of protein available for use by an animal (Launchbaugh, 2001; Frutos et al., 2004) which can cause significant weight loss in livestock. Besides their negative effects, tannins can enhance the flow of protein compounds through the rumen to the small intestines therefore escaping microbial fermentation (Launchbaugh, 2001; Waghorn and McNabb, 2003).

Besides the concentration of tannins in plant tissues, their biological effects in animals depends on many factors including their chemical structure, interaction with PSM and/or nutrients in the diet, plant growth stage, species and physiological condition of the animal (Makkar, 2003; Min et al., 2003). Tannins adversely affect palatability, intake and digestibility of forage species (Goel et al., 2005). On the other hand, small amounts of tannins are beneficial, explaining why they are not completely avoided by grazing animals (Jansen et al., 2007; Egea et al., 2014). Tannins protect dietary protein from ruminal digestion until it reaches the more acidic abomasum and small intestines, hence improving the protein nutrition of ruminants (Barry et al., 2001; Min et al., 2005). This promotes a more effective immune response (Niezen et al., 2002) and improves reproductive efficiency (Min et al., 2001).

When tannins are ingested in low to moderate concentrations, they can prevent bloating by binding cellular complexes produced during mastication (Launchbaugh, 2001; Frutos et al., 2004). Tannins can also protect animals against infestations by controlling the nematode

population or diseases caused by parasitic worms leading to higher average daily gain in growing animals (Launchbaugh, 2001, Niezen et al., 1998; Addisu, 2016).

Goats have the ability to counteract the negative effects of tannins through the secretion of tannin-binding salivary proteins, making them inactive (Launchbaugh, 2001). Goats are often not affected by poisonous compounds or anti-nutritional factors because they prefer to consume a variety of plant species, hence the detrimental effects of poison compounds found in certain plants are diluted (Luginbuhl et al., 2010). By so doing they eliminate foaming properties of forage legume proteins and reduce the rate of gas production during fermentation.

Management strategies to reduce the negative impacts of PA include drying before feeding, diluting browse with grasses and use of polyethylene glycol (PEG) (Waghorn and McNabb, 2003). PA bind indiscriminately to protein and easily create a protein (N) deficiency especially if poor-quality grasses make a substantial proportion of the diet (Waghorn et al., 1999b). Ruminants are tolerant of dietary PA and although it is apparent no studies have been done comparing different species, giraffes (*Giraffa camelopardalis*), goats and deer are more tolerant of dietary PA than sheep or cattle because their high-proline salivary proteins reduce its effect in the rumen (Waghorn and McNabb, 2003).

### **2.11.2 Oestrogenic compounds**

Some legumes (e.g. subterranean and red clovers) contain oestrogenic compounds like isoflavones (e.g. formononetin, deidzein, genistein and biochanin A), which may interfere with cattle reproductive performance, although this effect is likely to be of more practical importance in sheep (Holmes et al., 2007). Lucerne contains coumestans, which are oestrogenic compounds that may cause reproductive problems more commonly in sheep than cattle presumably because

cattle are less likely to be grazing large quantities of lucerne at mating time in spring (Holmes et al., 2007).

## **2.12 Palatability**

Palatability refers to those characteristics of a feed that elicit a sensory response in the animal and is considered to be the corollary of the animal's appetite for the feed (Baumont, 1996). Palatability is determined by a complex interaction between animal, plant and environmental factors (Heady, 1964; Marten, 1978). Palatability is a conditional plant characteristic that is dependent on animal species and their physiological status (Horadagoda et al., 2009). Relative palatability shows the ranking of forages to which the animal has access and is a means of preliminary identification of preferred species, so as to manage a mixed species sward of forages, favoring one species over others. Hence knowing forage preference is crucial in controlling pasture species balance (Horadagoda et al., 2009).

Plants vary in palatability and subsequently their preference by animals. The higher the palatability indices of a feed the higher its consumption by an animal but it does not necessarily enhance animal performance. Grazing animals consume a variety of forage species, but habitually have a preference over some and avoid others when they are given free choice. Palatability is a function of taste, odor texture and post-ingestion effects on animal (Provenza, 1995a). Preference for a particular feed is an animal behavioral trait and is attributed to several factors including forage palatability, associated plant species, and species of animal involved (Lambert et al., 1989; Mayland and Shewmaker, 1999).

Preference under pasture grazing was influenced by relative species abundance in dairy cattle (Rutter et al., 2004) and in sheep (Parsons et al., 1994). Illius et al. (1999) argued that goats in their study selected diets that tended to maximize intake rates (i.e., preference of a herbage



was based on how fast they could eat it). A diurnal pattern to preference was observed (Rutter et al., 2004; Parsons et al., 1994), with red clover predominating in the morning and preference for grass increasing during the day. It has been surmised that the diurnal feeding pattern may have evolved as an antipredatory feeding strategy (Penning et al., 1998) which minimizes the need for feeding at night by grazing forage with a slower rate of digestion and a lower passage rate (i.e. grass is grazed preferably in the evening because it has lower passage rate relative to red clover).

Rutter et al. (2004) hinted on a possible connection between dietary preference and rumen function. This was based partly on the observation by Merry et al. (2002) that the *in vitro* efficiency of microbial protein synthesis from mixtures containing different proportions of red clover and grass silages was highest with 70% red clover and 30% grass; and on the study by Rutter et al. (2004) which showed an overall preference of 73.8% of red clover to 25.4% grass.

Preference for herbage is also relative rather than absolute as was observed that both cattle and sheep chose to eat mixed diets when given a choice of red clover and grass (Rutter et al., 2004), even though greater intakes could have been realized eating only red clover. It is plausible the animals preferred a mixture so as to achieve nutrient balance or continuity of energy supply. Inclusion of forage legumes in the diet can also improve the palatability of feed and enhance feed intake. Some legumes are highly digestible, therefore they are capable of making nutrients available to rumen microorganisms. Normally, forage legumes used either as silage or grazed herbage lead to higher intake and animal production than grass silages of comparable digestibility (Dewhurst et al., 2009).

Animals tend to select good quality feeds and show little preference for feeds low in nutrients using their sight and sense of smell to detect delicate differences in feeds (Provenza,

1996; Baumont 1996). This preferential grazing can affect forage quality, persistence of certain species and increase of undesirable plant species in a pasture (Hoveland, 1996).

### **2.12.1 Factors affecting palatability**

Palatability is a complex phenomenon regulated by animals, plant materials as well as the environment factors. Goats and sheep feed on a wide variety of feeds, selecting those that meet their nutritional requirements and avoiding those causing toxicosis (Provenza, 1995a). Selection of feeds depends on both plant and animal factors. Among the plant factors influencing palatability are the ability of forage to provide stimuli to the oropharyngeal sense of the animal, plant species, intraspecific variation, chemical composition (e.g. sugar level, protein and lignin content, aroma and anti-nutritional values), texture, morphology or physical traits (awns, spines, hairiness, stickiness), succulence or maturation and form of the forage (Marten, 1978; Baumont, 1996; Hoveland, 1996; Mayland and Shewmaker, 1999).

Chemical compounds known to alter palatability and intake irrespective of the nutritional value of the feed include phenolics, alkaloids, tannins and aromatic compounds (Heady, 1964; Mayland and Shewmaker, 1999; Marten, 1978). A study on the palatability of six different rye grass varieties by cows showed that water soluble carbohydrates (WSC) was strongly correlated with palatability (Smit et al., 2006). Bitterness in plants is due to nitrates; in extreme cases nitrates can be toxic. Consequently grazing animals tend to avoid plants with high nitrate concentrations (Provenza et al., 2007). The fibre content of a plant, particularly the extent of lignification impacts the tensile strength (Baumont et al., 2000); animal preference is for those forages with a lower tensile strength since it makes prehension easier and intake rate higher (Inoue et al., 1989; Baker et al., 1994).

Animal factors known to influence palatability include sense, species or breeds, individual variation, previous experience and physiological condition (Marten, 1978). Variables within animal body include nerve stimuli, the senses, and others (Heady, 1964). The most important food cue influencing palatability is flavour (taste and odour) (Garcia, 1989); herbivores can detect toxic compounds by smell before eating or soon after the first bite (Personius et al., 1987).

Natural and induced environmental factors that often influence plant selection by ruminant animals include: plant disease, soil fertility, animal dung, feed additives, climate variation and seasonal or diurnal variations (Marten, 1978). In a stall-feeding experiment conducted in Minnesota, Marten (1978) showed the influence of the presence and absence of plant disease on apparent palatability of two forage species. The heifers consumed an average of 1.1 kilogram dry matter of brome grass which was heavily infested with *Helminthosporium* leaf spot compared to 1.7 kilogram of reed canarygrass during a 20-minute exposure (Marten, 1978).

Although palatability affects forage utilization by grazing livestock, relative palatability of native legumes is unknown; this study therefore investigated relative palatability of four (4) native legumes relative to Lucerne which is the predominant legume used for feeding livestock in Namibia. Aderibigbe et al. (1982) assert that the best way to evaluate palatability is to measure consumption of two or more forage offered simultaneously.

### **2.12.2 Nutrient imbalances and their influence on palatability**

Exhibited food preferences that may reduce feed intake in animals may be an attempt to avoid nutrient imbalances (Stevens et al., 2004). Kyriazakis and Oldham (1993) for example obtained peak voluntary feed intake at between 141 and 172 g/kg of DM CP when feeding lambs with paired choices of diets in the range of 78 to 235 g/kg of DM CP. Lambs facing protein

restriction appear capable of sensing dietary nutrient imbalances as they increase preference for a feed high in CP or a flavor (umami) that signals the presence of CP in feed (Naim et al., 1991). This behavioural change may be an attempt to fulfill the protein requirements after sensing the deficit (Bach et al., 2012).

Rutter et al. (2004) reported that cattle and sheep on a perennial ryegrass/white clover pasture both ate mixed diets, but showed a partial preference of about 70% for clover. The preference for white clover may be driven by the animal's desire to maximize nutrient benefit achieved per unit energy expended on grazing. Despite the preference for white clover, animals maintained a mixed diet, which may be related to the desire to maintain effective rumen function.

### **2.12.3 Diet choice in grazing animals in relation to palatability**

Models of foraging such as the optimal foraging theory (OFT) assume consumer decisions are based on the intrinsic properties of food including nutrient concentration and abundance (Villalba et al., 2015). In the classical OFT, animals maximize the long-term rate of energy intake (Stephens and Krebs, 1986) and on the basis of estimated profitability (fitness gain per investment of time), a food is either always or never taken. Newer models based on observations in generalist herbivores grazing diverse plant communities, take cognizance of partial preferences (Provenza, 1996; Provenza and Villalba, 2006). Partial preferences have been attributed to limited perception (Berec and Krivan, 2000), inability to discriminate (Illius et al., 1999) or sort (Courant and Fortin, 2010) among different plant species, attempts to meet nutritional needs (Westoby, 1978), or to dilute consumed plant secondary metabolites (PSM) (Freeland and Janzen, 1974).

Contrary to these restricted views, foraging behavior is affected by the consumer's past experiences with the biochemical context in which a food is ingested, including the kinds and amounts of nutrients and PSM in a plant and its neighbours (Villalba et al., 2015). Additionally, past experiences with food can potentially influence food preference through the mechanism of food hedonics (Villalba et al., 2015). Hedonic perception pertains to pleasant or unpleasant feelings evoked by the feed. To some degree, mechanisms of brain reward can induce hedonic feeding behavior in competition to physiological factors controlling intake (Baumont et al., 1996). The degree to which hedonic feeding behavior is elicited will depend on the palatability of feed. When good quality forages are fed to wethers, observed voluntary feed intakes greatly in excess of requirements can be explained by hedonic behavior (Baumont et al., 1989).

Consumed forages do not act in isolation in a herbivore's body (Villalba et al., 2000), but rather evolve into associative or antagonistic relationships that yield fitness benefits different from those of singly ingested foods (Tilman, 1982). These relationships interact with the herbivore's physiology, contributing to experiences that modify future decisions made when grazing diverse plant communities. Animals modify their foraging behavior based on learning experiences that emanate from the taste of food with post-ingestive feedback from cells and organs in response to levels of ingested chemicals (Villalba et al., 2015).

Consumed foods may interact, enhancing or reducing digestibility (Van Soest, 1994). When sheep or goats ate mixed diets on rangeland their daily intake were much greater than their contemporaries fed single forages of similar nutritive value under confinement (Agreil and Meuret, 2004; Meuret and Provenza, 2014a). A mixed diet permits the foraging animal to obtain a diet higher in nutrients (Westoby, 1978) that may dilute or inactivate PSM (Freeland and Janzen, 1974; Catanese et al., 2014), hence enabling improved growth and reproduction (Rapport, 1980;

Pennings et al., 1993). This study aimed at establishing preference or palatability of different forage legumes under a cafeteria system under a confined environment so as to shed light on potential utilization of these forages under grazing or pen conditions.

Extrapolation from pen trials to field tests for preference test has not always been possible and this is explained by the way these tests change diet selection. Offering the same food in different flavours in free-choice tests (e.g. Freidin et al., 2011), or foods of varying quality (e.g. Catanese et al., 2010) may not be a full representation of the types of foraging challenges animals face on the range. Free-choice tests commonly involve offering foods in a small area *ad libitum*; in nature food choice involves plants of diverse nutritional composition and variable temporal and spatial distribution (O'Reagain and Schwartz, 1995). In natural settings, herbivores will be sensitive to these sources of variation and adapt their intake rate and diet selection based on optimization rules that involve food quality and availability (Parsons et al., 1994b).

Under free-choice trials in pens it may not be possible to reveal how herbivores value a given low-quality food on the basis of their previous experience, because the costs of foraging are negligible. In natural conditions when conditions, however, change for instance preferred alternative forages decline in abundance, the associated travelling and handling costs increase, previous experiences eating low-quality foods by herbivores come to light (Villalba et al., 2015). This may have implications in grazing management by way of integrating preference conditioning procedures to improve experience with low quality forages and environmental conditions to encourage their use under natural situations (Villalba et al., 2015). Thus results obtained from the preference tests that were conducted in this study should be treated as

indicative of trends, subject to post-ingestive effects and availability of forage legumes in both time and space.

### **2.13 Voluntary intake of forage**

The voluntary intake of feed is defined as the *ad lib* intake of an animal when offered an excess of a single feed or forage (Van Soest, 1985). Voluntary intake is one of the measures used in assessment of forage quality (Deinum, 1984). The voluntary intake of different forage types differs: intake of legumes is often greater than grasses and this is attributed to the legumes containing less cell wall than grasses and they have lower retention time in the rumen (Meissner et al., 2000). In many grasses the sclerenchyma tissue above and below the vascular bundles facilitates the stronger attachment of the cuticle and epidermis to the interior tissues, making grasses more resistant to physical digestion mechanisms (Pond et al., 1984)

Feed intake in cattle is influenced by the eating rate, rumen fill and metabolic control (Richardson and Smith, 2006). The dominating factor in limiting intake will, however, depend on nutrient demand (Voelker Linton and Allen, 2008). Cattle consume taller or denser swards more compared to short sparse ones, since more herbage can be taken in with each bite. Under selective feeding, for example when there is a low proportion of leaves to stems, bite size will also be reduced (Richardson and Smith, 2006).

#### **2.13.1 Physical constraints to voluntary feed intake in ruminants**

##### **2.13.1.1 Rumen fill and passage rate effects on Voluntary dry matter intake**

Voluntary dry matter intake (VDMI) may be limited in ruminants consuming forages as a result of restricted flow of digesta through the gastro-intestinal tract. Due to the restricted flow, distention in one or more segments of the gastrointestinal tracts may occur, which reduces intake

(Allen, 1996). Since the suggestion that ballast of undigested feed residues in the gastrointestinal tracts may limit intake (Lehman, 1941), there has been mounting evidence to support this theory (Campling, 1970; Forbes, 1995).

VDMI of forages by sheep is more highly related to NDF than to other chemical measures (Van Soest, 1965). Waldo (1986) reached a similar conclusion and suggested NDF as the best single chemical predictor of VDMI by ruminants. In spite of NDF being used as the sole feed characteristic to predict the filling effects of forages (Mertens, 1987; Mertens, 1994a), evidence suggests NDF alone is inadequate; its filling effect varies with differences in initial particle size, particle fragility and rate and extent of NDF digestion. Moreover the fill effects of the fiber depends on several characteristics such as the dimensions of the indigestible and potentially degradable fractions and the rate of removal from the rumen (Waldo et al., 1972; Detmann, 2010)

VDMI is related to digestibility of forages (Blaxter et al., 1961; Blaxter and Wilson, 1962; Van Soest, 1965). This relationship, however, is not linear, with decreased response in VDMI as digestibility increases (Blaxter et al., 1961). Physical distention in the gastrointestinal tract is believed to restrict VDMI of low digestibility feeds, which it is suggested diminishes as digestibility increases. NDF content and DM digestibility explain similar variation in VDMI of forages; VDMI decreases with an increasing negative slope as forage NDF content increases, such a decrease is consistent with the theory that fiber mass in the gastrointestinal tract may inhibit VDMI (Van Soest, 1965). On less fibrous diets that are nutrient rich (e.g. high concentrate diets), metabolic feedback will contribute to satiety.

In the gastrointestinal tract, the reticulorumen is regarded as the site at which distension limits intake with high-fill diets (Campling, 1970; Baile and Forbes, 1974). Located primarily in



the reticulum and cranial sac are tension receptors which respond to distension (Leek, 1986). Grinding and pelleting of forages to decrease particle size in general increases VDMI (Minson, 1963), as a result of a reduction in initial volume and retention time in the reticulorumen (Moore, 1964). Pelleting of low-quality forages gives a greater intake response than that of high-quality forages (Minson, 1963) because fill limits VDMI to a greater extent for forages with low digestibility and high fiber contents.

The most important factor in predicting intake and digestibility is retention time (Mertens, 1973). Digestibility can be depressed at high intakes (Raymond et al., 1959; Alwash and Thomas, 1974; Tyrell and Moe, 1975). In milking cows, digestibility declined linearly with increasing metabolic requirements (Tyrell and Moe, 1975). Retention times in all diets fed to sheep decreased with increased intake and the reduced digestion was correlated with the reduced retention time (Alwash and Thomas, 1974). In addition, particle size was positively correlated with retention time and digestibility (Demment and Van Soest, 1985). Mertens (1973) concluded that passage rate is a more important determinant of rumen fill than digestion rate. Passage rate can be increased by increasing the rate of particle break down (which occurs by the processes of rumination and digestion) or by increasing the size of the particles which escape the rumen (Demment and Van Soest, 1985).

Many experiments (Campling et al., 1961; Freer and Campling, 1963; Thornton and Minson, 1972, 1973; Laredo and Minson, 1975) have demonstrated the inverse relationship between retention time in the reticulorumen and VDMI. In a study with sheep (Jung and Allen, 1995), higher NDF intakes were observed for alfalfa hay than oat hay and this was attributed to a lower filling effect of the alfalfa NDF due to a faster rate of digestion and passage from the reticulorumen.

The reticulorumen is assumed to be the primary control point for physical limitation to intake, with capacity proportionate to body weight (BW). Gut content is highly related to BW for herbivores (Demment and Van Soest, 1983). In 18 species of ruminants in the weight range 3.7 to 720 kg, the DM in the rumen was highly correlated ( $r^2 = .98$ ) with BW (Illius and Gordon, 1991). Capacity for fill in the reticulorumen is also affected by the animal's physiological state. Volume of rumen contents of sheep on a hay diet decreased as pregnancy progressed (Forbes, 1969). Both weight and volume of reticulorumen contents affect fill because tension receptors are stimulated by their combined effects (Allen, 1996). Weight is an important determinant of fill; this is supported by the observation that VDMI of fresh forages is limited by moisture content (Davies, 1962).

Fractional passage rate from the reticulorumen increases as the DMI increases (Riewe and Lippke, 1970) and during cold stress (Kennedy et al., 1976). In a trial involving Hereford x Angus cows consuming chopped hay from 61 to 6 d before calving, VDMI increased, fill in the reticulorumen decreased and fractional passage rate of indigestible ADF increased by 87% (Stanley et al., 1993). Efficiency of digesta flow per contraction increased at higher intakes (Deswysen and Ellis, 1988).

### **2.13.1.2 Fermentation rate effects on voluntary dry matter intake**

The digestibility of the diet decreases with increased feed intake. Reduced digestibility is primarily attributed to decreased residence time in the rumen, which does not permit complete digestion of digestible NDF (DNDF) (Huhtanen et al 2006). Reduced rates of NDF digestion occur when DM intake increased (Staples et al., 1984; Robinson et al., 1987; Okine and Mathison, 1991). The adverse effect of intake on cell-wall digestion is due to increased VFA concentrations, with concomitant decrease in rumen pH (Tamminga and van Vuuren, 1988;

Volden, 1999), to which cellulolytic bacteria are sensitive. The reduction in OM digestibility with increased DM intake can largely be attributed to lower NDF digestibility (Llano and DePeters, 1985; Huhtanen et al., 1995; Volden 1999). In contrast, for some types of diets for example rations rich in slowly degradable maize starch, an increase in DM intake can also significantly reduce the digestibility of cell solubles (Colucci et al., 1982)

Reduction of large particles is a necessity to flow from the reticulorumen (Ewing, 1918). Rumination and mastication are the primary means of breakdown of large particles (Kennedy, 1985); relatively little breakdown results directly from digestion and detrition (McLeod and Minson, 1988). Insignificant reduction in size occurs after particles leave the reticulorumen (Poppi et al., 1980; Okine and Mathison, 1991a). On its own, fermentation has minimal direct effects on particle size reduction (Murphy and Nicoletti, 1984; Wilson et al., 1989b), none the less it affects rate of particle size reduction during chewing by increasing tissue fragility (Chai et al., 1984). As particle size increases, resistance to flow from the reticulorumen increases (Poppi et al., 1980; Dixon and Milligan, 1985). Animal size is thought to have a bearing on the rate of particle size breakdown, because rumination time per gram of NDF decreases exponentially with BW (Welch, 1982).

In general, grasses have higher fractions of potentially fermentable fiber and lower rates of fermentation than legumes (Smith et al., 1972), hence they tend to be buoyant for a longer time (Allen, 1996). Legume forages are thought to have faster rates of particle size reduction and rate of increase in particle specific gravity than grasses (Voelker Linton and Allen, 2008). Legume forage particles are more fragile than grasses and this has been specifically demonstrated in alfalfa (Chai et al., 1984; Waghorn et al., 1989). Hence both rate of NDF digestion and rate of particle size reduction usually are greater and time they are retained in the

rumen shorter in legumes than grasses (Waghorn et al., 1989; Hoffman et al., 1993; Holden et al., 1994). Moreover, particle density is thought to increase more slowly in grasses than legume particles because of more slowly digested potentially digestible NDF(pdNDF) in grass compared to alfalfa fibre (Allen, 1996). In this study, it was of interest to determine how voluntary intake would be affected through supplementation with legumes with potentially higher fibre digestion and also that could boost rumen microbial activity through supplying required N.

Prediction of VDMI is important in forage assessment. More than 50% of the variation in digestible nutrient consumption can be attributed to differences in VDMI. Restricted flow of digesta through the gastrointestinal tract results in distention, reducing VDMI particularly with declining forage quality (Allen, 1996). There is evidence of additive effects of physical and chemical stimuli on VDMI (Mbanya et al., 1993) and more research is needed on how both are integrated. This study was aimed at generating preliminary information that could be useful in forage assessment at least as far as impacts on VDMI and growth rate are concerned; refinements could then be effected in ration formulation based on the most promising feed ingredients.

### **2.13.2 Metabolic constraints to voluntary feed intake in ruminants**

The most important factor affecting animal performance is food intake (Illius and Jessop, 1996), yet the prediction of intake is the weak point in all current methods or models of diet formulation. Physical constraints relate to gut fill, whereas metabolic constraints are related to the animal's ability to utilize absorbed nutrients; both physical and metabolic constraints affect intake of both highly digestible and low quality forages (Illius and Jessop, 1996). Research has indeed shown that intake of poor quality forages could be enhanced by improving supply of protein relative to energy in the supply of absorbed nutrients (Egan, 1977). In their conceptual

model, Illius and Jessop (1996) have argued that the optimal protein:energy ratio is markedly influenced by the biological value of the absorbed amino acids. This in turn is influenced by the microbial activity, composition of bypass protein and by the aggregate pattern of amino acids required by the animal's tissues.

In their model, when protein:energy ratios are below the optimum, compensating increases in food intake are predicted to result in increased lipid synthesis due to excess energy intake. In contrast, if protein:energy ratios are greater than optimal, increased deamination provides energy reducing the additional requirement for energy to match protein intake. The increased losses of N may be economically and environmentally undesirable (Illius and Jessop, 1996; Peyraud et al., 2009). Nutrient imbalances are presumed to constrain intake because of accumulation of excess metabolite. Nutrient imbalances can also lead to sensations of discomfort which cause reduced intake or avoidance (Provenza, 1995). It appears animals rely on these sensations and post-ingestion feedback mechanisms to avoid nutrient imbalances and toxins and to modify nutrient intake if they are given a chance to choose from a wide range of feeds (Burrit and Provenza, 1992; Provenza et al., 1994).

This study was aimed at providing preliminary information on the biological value of the absorbed amino acids because the different legumes likely have different influences on microbial activity, rate of degradation and antinutrients. Ultimately, it may be possible that combinations of legumes could be used to formulate diets with correct nutrient balance, giving maximal performance for a given production function (growth, lactation or reproduction).

Components in the diet have different effects on rumen microbes and interactions between different dietary components in rumen digestion can occur (Huhtanen et al., 2006). The rate and extent of digestion of low quality forages can be restricted due to a deficiency of supply

of essential nutrients for example N, S or in some cases branched-chain VFA (Hoover, 1986). On the other hand, in high producing ruminants fed mixed diets, the rate of cell wall digestion specifically can be retarded by substrates that inhibit the growth of rumen cellulolytic bacteria.

When the concentration of non-structural carbohydrates (mainly starch and sugars) is increased in the diet, there is usually a decrease in fibre digestion (Huhtanen et al., 2006). The lower rumen pH that results from increased supply of nonstructural carbohydrates decreases the rate of cell wall digestion, due to cellulolytic bacteria being more sensitive to low pH than those utilizing starch (Hungate, 1966; Russel and Dombrowski, 1980). The effect of rumen pH on *in vitro* cell wall digestion varied with substrate (Grant and Mertens, 1991). Legume digestion was less sensitive to lower rumen pH than that of grasses (Grant and Mertens, 1991), possibly because of the higher buffering capacity of legumes. This suggests that in feedlot diets where high levels of concentrates are used, forage legumes especially those with low potential for causing bloat or other adverse effects, may have an important role to play. *In vitro* studies have shown that the activity of cell wall degrading enzymes depends on the carbon source (Groleau and Fosberg, 1981; Williams and Withers, 1982).

Digestion of the cell wall is reduced by limited N availability (Huhtanen et al., 2006). A minimum ammonia concentration of  $3.6 \text{ mmol L}^{-1}$  has been suggested when dietary CP exceeds  $60 \text{ g kg}^{-1} \text{ DM}$  (Hoover, 1986). Protein supplementation increases the rate of digestion of cell-wall carbohydrates of forages which contain less than  $80 \text{ g CP kg}^{-1} \text{ DM}$  (Ellis et al., 1999). The minimum amount of available N has been postulated to depend on the digestibility of forages and is relatively higher for highly digestible forages (Mertens, 1993a). The concentration of dietary N needed to optimize cell-wall digestion depends on the rumen protein degradability (Orskov, 1982). Protein supplementation improved digestibility of digestible NDF (DNDF)

which suggests that increased availability of amino acids in the rumen improved cell wall digestion that may have been mediated through increases in the rate of digestion (Shingfield et al., 2003).

Nutrient balance is important in the rumen and in the nutrients absorbed by the animal. Like in more complex organisms, there is an optimum nutrient balance for rumen microorganisms; failure to achieve this nutrient balance results in reduction in efficiency of conversion of nutrients into compounds that can be utilized by the host animal (Illius and Jessop, 1996). Asynchrony in the supply of nutrients to microbes results in serious negative impacts on the efficiency of substrate fermentation and on the rate of microbial growth (Beever, 1993). Restricted supply of ruminally available nitrogen relative to energy severely limits microbial growth and activity and may result in a reduction in rate of forage digestion (Leng, 1990). Similarly, restricted supply of fermentable carbohydrate relative to nitrogen limits microbial growth and activity and leads to inefficient use of amino acids because greater amounts will be deaminated, increasing the loss of nitrogen in the form of urine from the animal (Illius and Jessop, 1996).

Although there is need to supply the correct balance of fermentable nitrogen and carbohydrates to ruminal microorganisms so as to maximize rumen efficiency (Sniffen et al., 1992; AFRC, 1993), complications arise in the determination of the total nutrient supply at the rumen level due to the fact that ruminally available nitrogen arises not solely from the feed but also from endogenous sources (Leng and Nolan, 1984). Hence in supplying ruminally available protein in proportion to energy, diet fermentability or feeding frequency should be considered. At low intake levels, or under circumstances of low and fairly constant intake (e.g., grazing), any

asynchrony in dietary nutrients will be masked by endogenous inputs. Significant asynchrony would be observed only if this intake exceeds this nitrogen input (Illius and Jessop, 1996).

Additional metabolic mechanisms limiting feed intake include thermoregulation, growth potential and lactation (Richardson and Smith, 2006). In a tropical environment, high ambient temperatures limit heat dissipated to the environment and may restrict feed intake. Cows in lactation, have higher intake than dry cows of similar size; notwithstanding, the higher intake is unable to meet the metabolic requirements of cows in early lactation, leading to a negative energy balance.

The theory on the control of intake in ruminants is based on the integration of physical constraints and metabolic feedbacks which determines the maximum DMI for a specific animal under defined feeding situations (Crampton, 1957; Blaxter et al., 1961; Conrad et al., 1964). Hence for low quality diets intake would be constrained by physical limits of the gastrointestinal tract (GIT) to hold the bulk from diet. The DMI of animals on a high quality diets on the other hand, would be limited by metabolic feedbacks that would be responsive to dietary energy (Mertens, 1994). This approach has, however, been criticized because of its intrinsic assumption that both physical constraints and metabolic feedbacks are independent of each other (Detmann et al., 2014). Based on their model, Detmann et al. (2014) concluded that integration of physical constraints and metabolic constraints regulates voluntary feed intake.

#### **2.14 Summary of important characteristics of some of the legumes**

There is paucity of information on indigenous legumes of Namibia, hence only a limited account on a few of the forage legumes follows, based on the sources which were accessible. The morphology and growth characteristics of different indigenous forages (Adebisi and Bosch, 2004; Moteetee and Van Wyk, 2011) are shown in Table 2.1. The growth characteristics and



morphology were the basis for preliminary selection of legumes with potential for forage production that will be discussed in the subsequent chapters.

### **2.14.1 *Vigna* spp.**

*Vigna* genus belongs to the important legume tribe *Phaseoleae* (which also contains soybeans and the common bean *Phaseolus vulgaris* L.) and has undergone and still is a key topic in taxonomic shuffling (Schrire, 2005). All *Vigna* species are haerbaceous, both annual and perennial, some are climbing, and they often grow in soils that are of low fertility and well-drained (Schrire, 2005). Some of the species of *Vigna* (e.g. *V. heterophylla*, *V. luteola*, *V. vexillata* [common name *V. lobatifolia*]) are primarily valued for their tubers (Sprent et al., 2010). Besides being perennating organs, tubers enable plants to store nutrients (water inclusive) over dry periods and hence tuber crops are of importance in an increasingly drier and infertile environment (Sprent et al., 2010). During field excursions in central Namibia, *Vigna lobatifolia* was observed to flower shortly (1 – 2 months) after the end of the cold season (May to August), which may be an adaptive feature to produce seed early in the summer season for dispersal. *Vigna* species can be used for multiple roles including soil improvement, feed for humans, medicines and for animal feed (Sprent et al., 2010). In their native soils, all *Vigna* species apparently nodulate freely using mainly slow-growing *Bradyrhizobia*, but just like in the case of cow peas, potential exists to enhance both plant and rhizobial germplasms for optimizing nitrogen fixation (Sprent et al., 2010). *V. lobatifolia* was included in the study due to its relative abundance and field observations of goats consuming it in the camps at Neudamm campus farm.

### **2.14.2 *Crotalaria podocarpa***

The native herbaceous legume *C. podocarpa* is widespread in eastern and southern Africa (Polhill, 1968). *C. podocarpa* occurs as a pluvial-therophyte in moderate numbers after sufficient rains and has significantly increased in Namibia's escarpment region hence reducing biomass production and vigour of perennial grasses and permanently damaging grass tussocks (Fischer et al., 2015). This annual herb looks like a shrub; its height, diameter, flowers and seed set vary considerably depending on water availability (Fischer et al., 2015). Pods of *C. podocarpa* contain 14 seeds (10 -17 seeds pod<sup>-1</sup>) with a seed mass of  $21.3 \pm 0.3$  mg (Fischer et al., 2015). The *Crotalaria* species use explosive dehiscence for primary dispersal (Stamp and Lucas, 1990), although their inflated and balloon like pods suggest additional secondary dispersal by wind or rolling on the ground (Le Roux et al., 2011). It was included in the study because it was abundant in the Tsumis area and goats readily consume it (Januarie, E. personal comm.2014).

### **2.14.3 *Otoptera burchellii* DC.**

The genus *Otoptera* belongs to the tribe Phaseolinae (Fabaceae) (Lackey, 1981). There are only two species of *Otoptera*, one (*O. burchellii* DC.) in Southern Africa (Botswana, Namibia, South Africa and Zimbabwe) and the other (*O. madagascariensis* R. Vig.) which is endemic to South-West and West Madagascar (Lewis et al., 2005). *Otoptera* are climbing shrubs up to 0.5 m high and 0.8 m wide with leaflets lanceolate or narrowly triangular to ovate; fruits are linear, oblong, glabrous 60 – 120 x 3 – 6 mm, 4-seeded; seeds oblong, kidney-shaped, 7 – 8 mm long, dark brown (Moteetee and Van Wyk, 2011). The key difference between the two species of *Otoptera* is in the orientation and size of the spur at the base of the wing (Verdcourt, 2001).

#### 2.14.4 Lablab or hyacinth bean (*Lablab purpureus* subsp. *purpureus*)

Lablab (*Lablab purpureus* which has the synonymous name *Dolichos lablab* L. belongs to the family Papilionaceae. Lablab is a climbing or bushy perennial herb, often grown as an annual with a well-developed taproot, with many lateral roots. The stem is up to 6 m long, in cultivated plants often erect, pubescent or glabrous (Adebisi and Bosch, 2004). Lablab consists of a single species, but three subspecies have been described. Subspecies *uncinatus* Verdc. comprises wild plants occasionally cultivated in East Africa, with comparatively small fruits, c. 4 cm x 1.5 cm. Subspecies *bengalensis* (Jacq.) Verdc. is a short-lived perennial, twining herb grown as an annual with distinctive tender fruits up to 15 cm x 2.5 cm and is commonly found in tropical and subtropical regions of Africa, Asia and the Americas. Subspecies *purpureus* is a long-lived semi-erect bushy perennial, but is grown usually as an annual and show little or no tendency to climb; the fruits are relatively short, up to 10 cm x 4 cm (Adebisi and Bosch, 2004). The subspecies that were investigated in this study were *Lablab purpureus* subsp. *purpureus* and *Lablab purpureus* subsp. *uncinatus*; both species were sampled to determine chemical composition, but only *Lablab purpureus* subsp. *purpureus* was used in the voluntary feed intake experiment.

Lablab is used as a fodder for cattle either green or as hay or silage. Lablab is under investigation in Namibia for use primarily as a green manure crop (Shaningavamwe K. L., pers. Comm., 2016) although it has a high nutritive for feeding ruminants and is grown as an annual or short-lived fodder crop on a large scale in Australia, Kenya and Zimbabwe; in the latter two countries it is fed as a supplement to poor quality stover and hay (Adebisi and Bosch, 2004). The CP content in the dry matter of the whole plant is 10 – 22% while in leaves it is 14.5 – 38.5%; the leaves do not contain tannins (Adebisi and Bosch, 2004). After establishment (2 – 3 months

after sowing) Lablab is quite drought tolerant; fodder dry matter yields of 5 – 11 t/ha have been reported (Adebisi and Bosch, 2004; Tacheba and Moyo, 1988). Lablab was included in the experiments because of potentially high yields obtained in Namibia (Kaholongo, 2016) and due to its high nutritive value (Kanani et al., 2005).

#### **2.14.5 Lucerne (*Medicago sativa*)**

Lucerne is high in crude protein (CP) concentration (253 g/kg DM at pre-bud stage declining to about 171 g/kg DM at early flower stage), but has a low fermentable carbohydrate content (MacDonald et al., 1991, 2010) and hence exhibits a high buffering capacity. The high buffering capacity of Lucerne is advantageous because it supports a stable rumen pH that optimizes microbial activity (McBurney et al., 1983); in ruminant diets, lucerne often increases feed intake when compared with other forages (Bulang et al., 2006). Lucerne was included in the study because it is highly nutritious and is the predominant forage legume used in feeding livestock as hay in Namibia.

**Table 2.1: Plant growth habit and suitability status for foraging or fodder production**

Forage legume	Plant growth habit	Comment*
<i>Crotalaria argyraea</i>	Erect shrub	Browsed by goats
<i>Crotalaria heidmannii</i>	Erect shrub	Occasionally browsed
<i>Crotalaria podocarpa</i>	Erect shrub	Occasionally browsed
<i>Cullen tomentosum</i>	Prostrate	Ordorous and not liked by goats
<i>L. purpureus</i> <sup>1</sup>	Long-lived, semi-erect bushy perennial, showing little or no tendency to climb; fruits are relatively short, up to 10 cm x 4 cm	Suitable for fodder production
<i>L. purpureus</i> <sup>2</sup>	perennial, twinning herb; comparatively small fruits, c. 4 cm x 1.5 cm.	May be suitable for fodder production; could also be grazed <i>in situ</i>
<i>Meulobium candicans</i>	Short erect shrub; develops thorns late in the growing season	Could be browsed during early vegetative growth stage
<i>Medicago laciniata</i>	Prostrate with tiny leaves, forming a mat on the ground.	Low potential biomass production. Not suitable for grazing or browsing.
<i>Medicago sativa</i>	Erect	Grown commercially for fodder production
<i>Otoptera burchellii</i>	Semi-prostrate and forms stolons which easily form adventitious roots at the nodes.	Suitable for browsing and can also be harvested as fodder.
<i>Ptycholobium biflorum</i>	Perennial woody herb reaching 30 cm in height. Leaves simple, linear/lanceolate, slightly waxy. Pods are characteristically contorted.	Suitable for browsing
<i>Rhynchosia fleckii</i>	prostrate	Suitable for browsing
<i>Rhynchosia totta</i> (Thunb.)	Trailing, climbing and twining species	Suitable for browsing
<i>Vigna lobatifolia</i>	Trailing	Suitable for grazing <i>in situ</i> or fodder production

<sup>1</sup>*L. purpureus* is the cultivated lablab (*Lablab purpureus*) subsp. *purpureus*; <sup>2</sup>*L. purpureus* (L.) is *Lablab purpureus* (L.) sweet subsp. *uncinatus* verde. var. *rhomboideus* (Schinz) verde. (wild type lablab).

\* Based on our observations in the field of the plant growth habits for the native forage legumes.

<sup>1,2</sup>Adapted from Adebisi and Bosch (2004)

## CHAPTER 3

### 3 Determination of chemical composition and nutritive values of native forage legumes in comparison to *Medicago sativa* and *Lablab purpureus* (objective 1)

#### 3.1 Introduction

Generally legumes are known to have higher concentrations of CP and minerals compared to grasses, even when grown under similar conditions. The chemical composition of feeds is one of the important determinants of digestibility (Poppi et al., 1981b; Luginbuhl et al., 1994). Feeds that may appear to be similar can differ in digestibility on account of differences in chemical constituents which hinder digestive enzymes in getting in contact with their respective substrates. On the contrary, digestibility of complete feeds can be improved by the addition of relatively small quantities of specific nutrients for example protein or soluble carbohydrates (Khan et al., 2003).

Indigenous forage legumes have the potential to improve the nutritive value of ruminants, but there is limited information on their morphological characteristics and chemical composition. The objective of this research was to determine the chemical composition (CP, ADF, NDF, CF, EE, ash, Ca and P) and estimate the metabolizable energy (ME) of the native forage legumes *Crotalaria argyraea*, *Crotalaria heidmannii*, *Crotalaria podocarpa*, *Cullen tomentosum*, *Lablab purpureus* (L.) sweet subsp. *uncinatus* verd. var. *rhomboideus* (Schinz) verde (hereafter wild type lablab), *Meulobium candicans*, *Medicago laciniata*, *Otoptera burchellii*, *Ptychlobium biflorum* (E. Mey.) Brummitt (hereafter *Ptychlobium biflorum*), *Rhynchosia fleckii* Schinz (hereafter *Rhynchosia fleckii*), *Rhynchosia totta* (Thunb.) DC. var. *totta* (hereafter *Rhynchosia totta*) and *Vigna lobatifolia* in comparison to lablab or hyacinth bean (*Lablab purpureus*) (with

the synonymous name *Dolichos lablab*) and Lucerne (*Medicago sativa*), which are some of the legumes used in ruminant diets.

## 3.2 Materials and Methods

### 3.2.1 Collection of legumes

Forage legume samples were obtained from the wild by identifying plants that had three leaflets and/or with root nodules during the growing season (October to May) which were sent to the National Botanical Research Institute (NBRI) for identification. Subsequently, forage legumes were collected from the wild in central Namibia (primarily from Neudamm campus farm, road sides and Tsumis arid zone agricultural center [TAZAC]) during the rainy season (January-April in 2014 and 2015). The average annual rainfall of Neudamm campus farm is 350 mm; TAZAC has annual rainfall of 150 mm. The legumes *Otoptera burchellii*, *Vigna lobatifolia*, *Rhynchosia totta*, *Medicago laciniata* and *Cullen tomentosum* were obtained from Neudamm campus farm. The legumes *Crotalaria argyraea*, *Crotalaria heidmannii*, *Crotalaria podocarpa* were exclusively from TAZAC; *Otoptera burchellii* was also obtained from TAZAC. *Cullen tomentosum*, *Meulobium candicans*, *Rhynchosia fleckii* were primarily found and harvested from road sides (Windhoek – Hosea Kutako Airport road; Windhoek – Rehoboth road); in addition substantial quantities of *Vigna lobatifolia*, *Otoptera burchellii* were harvested along road sides.

The samples were air-dried under a roofed house for a period of 2 – 3 weeks and then stored in paper bags until needed for chemical analyses. Representative samples of the legumes were obtained from separate batches of legumes harvested with each year in a given location. Twenty eight (28) and thirty seven (37) random samples of the legumes were obtained in 2014 and 2015, respectively. The average number of samples analysed for each legume was five (5). Grass hay and Lucerne (*Medicago sativa*) samples were obtained from different batches used for

the palatability and feeding trial experiment and that were sourced from a commercial supplier. For comparison, lucerne and Lablab (*Lablab purpureus* subsp. *purpureus*) samples were also obtained from field plots in a related study on evaluation of indigenous forages (Kaholongo, 2016) at Neudamm campus. *Lablab purpureus* (L.) sweet subsp. *uncinatus* verd. var. *rhomboideus* (Schinz) verde (hereafter referred to as wild type lablab) was obtained from a single camp at Neudamm campus farm and was air-dried in a roofed house and processed in the same way like other samples.

### **3.2.2 Laboratory protocols for nutrient analyses**

A representative sample from each forage species was ground to pass through a 2 mm sieve mesh using a Wiley Mill (Glen Creston Ltd, Middlesex, UK). Samples were then packed into individual clear plastic air-tight containers and taken to the Nutritional Laboratory in MAWF for analyses of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and proximate parameters.

Dry matter (DM), Ash, Crude Fiber (CF), Ether Extract (EE) and CP of feed samples were analysed using the procedures described in Association of Official Analytical Chemists (AOAC) (Horwitz and Latimer, 2005). While ADF and NDF analyses were based on the method described by Robertson and Van Soest (1981). All chemical analyses were carried out in duplicate and results are expressed on DM basis.

#### **3.2.2.1 Moisture determination**

DM is used to determine the amount of water present in a feed sample. Moisture content was determined by placing the ground feed samples (2 g) in cleaned crucibles and heating them overnight in a convention oven at 105°C. Empty crucibles were weighed to establish the weight



of each crucible; then the crucible and wet sample before placing the crucible in the oven. The samples in crucibles were transferred from the oven into a desiccator and cooled at room temperature. Finally, the weight of the crucible and dried feed samples was recorded. Moisture content is the difference between the initial weight of the samples and that of the final weight of the sample, whereas the final weight of the samples is the dry matter. The moisture content was estimated using equation (1):

$$\text{MC}(\%) = \frac{[(w_1 - w_0) - (w_2 - w_0)]}{(w_1 - w_0)} * 100 \quad (1)$$

Where:

MC (%) = moisture content of sample (MC %)

$W_0$  = tare weight of crucible (g)

$W_1$  = weight of empty crucible + wet sample (g)

$W_2$  = weight of empty crucible + dry sample (g)

$\therefore$  DM (%) = 100 – MC (%)

### 3.2.2.2 Ash content

The ash content of feed samples was measured by heating the samples (2 g) in the presence of oxidizing agents. Samples were heated in a muffle furnace at high temperatures of 550°C for 5 hours to remove water and organic substances. Samples were weighed before and after ashing to determine the amount of ash present. The ash concentration (%) was calculated using equation (2):

$$\text{Ash}(\%) = \frac{w_2 - w_0}{w_1 - w_0} * 100 \quad (2)$$

Where:

Ash (%) = ash content of the sample (%)

$W_0$  = weight of an empty dry crucible (g)

$W_1$  = weight of an empty dry crucible + dry sample (g)

$W_2$  = weight of an empty dry crucible + ash (g)

### 3.2.2.3 Crude protein (CP)

CP is calculated from the nitrogen (N) content of the feed sample. The N content of feed samples (2 g) was determined by total combustion according to Dumas procedures. Samples were combusted in high temperature (950°C) chamber in the presence of oxygen and Ethylenediaminetetraacetic acid ( $C_{10}H_{16}N_2O_8$ ) was used as reagent and the calibration standard.

A factor of 6.25 was used to convert the N concentration of feed samples to CP content as shown in equation (3):

$$\% \text{ CP} = \% \text{ N} \times F \quad (3)$$

Where:

% CP = Crude protein percentage

% N = Percent Nitrogen automatically calculated in combustion apparatus

F = 6.25 (the average nitrogen content of protein)

### 3.2.2.4 Ether extract (EE)

Ether extract (EE) was determined using petroleum ether (FAO, 2011). Three grams of each ground sample was weighed in individual extraction thimble in a Solvent Extractor. A firm cotton wool plug was placed in each thimble in order to keep the sample in place during extraction. Empty, cleaned and dried Soxhlet flasks were weighed, then filled to about 2/3 of their capacities with petroleum ether and connected to the extraction apparatus. Extraction thimbles with samples were then placed in the extractor and properly sealed. The cooling water

supply and heating mantle were turned on and the temperature of the heating mantle was adjusted to 100°C for a period of 4 hours. Thereafter the thimble was removed and ether was distilled into a collection tube for re-use. The flasks were removed from the apparatus and placed in a cold, explosion proof oven for 1 hour at 105°C, then removed, cooled in a desiccator and weighed.

Then percentage of EE was calculated using the equation (4) below:

$$EE (\%) = \frac{MFR - MF}{M} \times 100 \quad (4)$$

Where:

MF = mass of flask (g)

MFR = mass of flask with extracted residue (g)

M = mass of sample used (g)

### 3.2.2.5 Crude Fiber (CF)

CF was determined by acid-base digestion method using 1.25% H<sub>2</sub>SO<sub>4</sub> (w/v) and 1.25% Sodium hydroxide (NaOH) (w/v) solution. One gram of ground feed samples were boiled in a weak acid of 0.128 M of Hydrochloric acid and thereafter samples were placed in a weak base (0.313M) of Sodium hydroxide. Subsequently the same sample was subjected to higher heat in a muffle furnace at 550°C for five (5) hours and then cooled down. CF content was measured by expressing the loss in weight after deducting the amount of ash as a percentage of the original weight of the sample, using the equation (5):

$$CF (\%) = \frac{W_2 - W_3}{W_1} * 100 \quad (5)$$

Where:

CF (%) = Crude fiber (%)

$W_1$  = sample weight (g)

$W_2$  = weight of the crucible + dry residue (g)

$W_3$  = weight of the crucible + Ash (g)

### 3.2.2.6 Acid-detergent fiber (ADF)

ADF was determined according to the procedures described by Robertson and Van Soest (1981), using Acid Detergent Solution (Cetyltrimethylammonium bromide- $C_{19}H_{42}BrN$ ) in 5 dm<sup>3</sup> of 0.5M Sulphuric acid and Pepsin-acid solution in 0.075M HCL. Two (2) grams of each feed sample were boiled in the Acid Detergent Solution (ADS) in a fiber extracting apparatus for 30 minutes. ADF solutions in crucibles were filtered out by suction, washed with warm distilled water and then rinsed using acetone. Samples was dried in a force draught oven at 105 °C and allowed to cool in a desiccator for 30 min before it was weighed again in order to obtain the second weight ( $W_2$ ). Thereafter, samples were ashed in the furnace at 550 °C for 5 hours and cooled down at a temperature below 250 °C before being transferred to a desiccator for 30 min to allow thorough cooling. Samples were further weighed to determine the third weight ( $W_3$ ). ADF was then measured by expressing the loss in weight after deducting the amount of ash, as a percentage of the original weight of the sample, as indicated in equation (6):

$$ADF(\%) = \frac{W_2 - W_3}{W_1} * 100 \quad (6)$$

Where:

$W_1$  = weight of original sample (g)

$W_2$  = weight of residue in crucible after drying (g)

$W_3$  = weight of residue in crucible after ashing (g)

### 3.2.2.7 Neutral-detergent fiber (NDF)

NDF was determined according to procedures described by Robertson and Van Soest (1981) using Neutral Detergent Solution (NDS), 2-ethoxyethanol Di-sodium-EDTA, Sodium borate decahydrate, Di-sodium hydrogen phosphate and  $\alpha$ -amylase, type X1-A). Samples were prepared in a similar way as in the procedures described in ADF determination. NDF percentage of the samples was then calculated using equation (7):

$$ADF(\%) = \frac{W_2 - W_3}{W_1} * 100 \quad (7)$$

Where:

$W_1$  = weight of original sample (g)

$W_2$  = weight of residue in crucible after drying (g)

$W_3$  = weight of residue in crucible after ashing (g)

### 3.2.2.8 Macro-minerals determination: phosphorus and calcium

The total amount of phosphorus and calcium in the feed samples was determined through dry ashing procedures in which samples were digested using 6 M of HCL and 6 M of Nitric acid ( $HNO_3$ ) reagents. Dried samples (2 g) were placed in porcelain crucibles and ashed in a muffle furnace at 550°C for 5 hours and allowed to cool down. After cooling down the samples were heated again in 5 cm<sup>3</sup> of 6 M of HCL for 2-3 hours in order to dissolve them. Samples were then cooled again and 6M of Nitric acid ( $HNO_3$ ) was added. The crucibles were removed immediately after the solution started to boil and filtered through the filter papers into 100 ml volumetric flasks. The filtered solution was then diluted using de-ionised water and thoroughly mixed and minerals were analysed using ICP Spectrometer (icap 6000 series).

### 3.2.3 Estimation of Metabolisable energy (ME)

Equations were used for estimation of metabolisable energy (ME) which predict total digestible nutrients (TDN) from the NDF content of forages (Undersander et al., 1993). Metabolisable energy (ME) was estimated assuming 1 kg TDN yields 4.4 MCal of digestible energy (DE) and ME is 82% of DE (NRC, 2001). The specific equation used for legumes was:

$$\text{TDN (\%)} = 86.2 - (0.513 \times \% \text{NDF})$$

For comparison, the TDN for the grasses used in the palatability and feeding trials was computed using the equation:

$$\text{TDN (\%)} = 105.2 - (0.667 \times \% \text{NDF})$$

### 3.2.4 Statistical analyses

The legumes were analysed for dry matter (DM), crude protein (CP), acid detergent fibre (ADF), Neutral Detergent Fibre (NDF), Ether Extract (EE), crude fibre (CF), ash, Ca, P, Total Digestible Nutrients (TDN), Digestible Energy (DE) and Metabolizable energy (ME). Logarithmic data transformation was carried out for EE and Ca to obtain a normal distribution; A square root transformation was carried out for CP. Data on ash underwent a reciprocal transformation to obtain a normal distribution. The same transformations stabilized variances on the respective variables. After transformation and analysis, the resulting least squares means were back-transformed and an error term for the original units was estimated using the untransformed data. Data from chemical analyses was analysed by GLM procedure (SAS, 2006); effects fitted in the models were legume species, location where it was harvested and year of harvest. Least squares means were separated by a PDIFF statement. Effects were considered significant at  $P < 0.05$ ; trends were accepted if  $0.05 < P < 0.10$ .

Residual analysis of CF showed moderate departure from the assumption of constant variance, but no suitable transformation could be found. Hence the Kruskal-Wallis non-parametric test was used and implemented using Proc NPAR1WAY (SAS, 2008). Comparisons of individual location (medians) differences for pairs of legumes were done using the same procedure, with the Wilcoxon option in the exact test (SAS, 2008).

### 3.3 Results

The univariate statistics on the different legume proximate variables are shown in Table 3.1. The DM was influenced ( $P < 0.05$ ) by legume species and the year in which it was harvested. The lowest DM (%) was observed in *C. heidmannii* ( $89.2 \pm 0.4$ ) and the highest was in *M. laciniata* ( $96.4 \pm 0.5$ ); lucerne had a DM of  $93.7 \pm 0.2$  and *L. purpureus* subsp. *purpureus* had a DM of  $92.8 \pm 0.3$ .

Table 3.2 shows the least squares means (%) for CP, EE, ADF, NDF, ash, Ca and P. The CP content was influenced ( $P < 0.05$ ) by legume species and the year when the harvesting was done. The CP (%) varied from a low value of  $12.3 \pm 2.1$  for *M. candidans* to the highest value of  $24.4 \pm 2.5$  for *C. podocarpa*. The legumes with the highest CP were *C. podocarpa*, *L. purpureus* subsp. *purpureus*, *M. sativa*, *O. burchellii* and *V. lobatifolia* and they did not significantly ( $P > 0.05$ ) differ. *M. candidans* had lower ( $P < 0.05$ ) CP than all legumes, with the exception of *C. heidmannii* with which there was no significant ( $P > 0.05$ ) difference and with *R. totta* (Thunb.) with which there was a trend ( $P = 0.0591$ ). The CP for all legumes was  $>20\%$  except for *C. heidmannii*, *L. purpureus* (wild type), *M. candidans*, *R. fleckii* and *R. totta* (Thunb.) DC. var. *totta*.

The ADF was influenced ( $P < 0.05$ ) by legume, location and year of harvest. The ADF of *M. candidans* ( $38.8 \pm 2.7$ ) exceeded ( $P < 0.05$ ) all other legumes except *C. tomentosum* and *R.*

*fleckii*. Similarly the ADF of *C. tomentosum* was higher ( $P < 0.05$ ) than all other legumes except *R. fleckii*. The legumes *O. burchellii*, *R. totta* (Thunb.), *V. lobatifolia*, *C. argyraea*, *L. purpureus* (wild type lablab) had intermediate ADF and it was similar ( $P > 0.05$ ) to that of Lucerne. *C. heidmannii* had the lowest ADF ( $16.3 \pm 3.2$ ). Some of the samples of Lucerne sourced from the commercial supplier had much lower ( $P < 0.05$ ) ADF than legumes sourced from other locations. The least square means of ADF for harvests in 2014 and 2015 were  $31.1 \pm 1.1$  and  $21.7 \pm 1.7$ , respectively.

The NDF was highly influenced ( $P < 0.0001$ ) by legume and year of harvest. NDF varied widely between the years ( $\text{NDF}_{2014} = 47.0 \pm 1.2$  vs.  $\text{NDF}_{2015} = 39.1 \pm 1.2$ ). The concentration of NDF was highest ( $P < 0.05$ ) in wild lablab, *M. candicans* and *C. tomentosum*; this was followed by *R. fleckii*, *R. totta* (Thunb.), *V. lobatifolia*, *O. burchellii*, *C. podocarpa*, *M. laciniata*, *L. purpureus*, *C. argyraea* and *M. sativa*; the lowest values were recorded for *C. argyraea*, *M. sativa*, *P. biflorum* and *C. heidmannii*. Dry matter was influenced ( $P < 0.05$ ; R-square = 87.7%) by legume species and place where it was harvested.

The EE was influenced ( $P < 0.05$ ) by legume, location and year of harvest. The EE was highest for *C. argyraea* ( $8.7 \pm 0.6$ ) and which differed ( $P < 0.05$ ) from all other legumes. The other legumes with moderate EE concentrations were *C. podocarpa*, *O. burchellii* and *R. totta* (Thunb.) DC. var. *totta* and these were not different ( $P > 0.05$ ). Samples harvested in 2015 had higher EE concentrations than those in 2014 ( $3.4 \pm 0.3$  vs.  $2.4 \pm 0.2$ , respectively).

The calcium concentration (%) was influenced ( $P < 0.05$ ) by legume, location and year of harvest. The highest Ca was in *R. totta* (Thunb.) DC. var. *totta* ( $4.2 \pm 0.6$ ) which was much higher ( $P < 0.05$ ) than all other legumes. The lowest calcium concentration was observed in *C. heidmannii*, *C. podocarpa*, *M. candicans* and *M. sativa*. Samples collected in 2014 had much



higher ( $P < 0.0001$ ) Ca concentration than those in 2015 ( $2.5 \pm 0.3$ , vs.  $1.1 \pm 0.4$ , respectively). Samples collected from TAZAC had higher ( $P < 0.05$ ) Ca concentration than those from road sides and Neudamm.

The phosphorous concentration (%) differed ( $P < 0.05$ ) among legumes and also location where the legume samples came from. The highest P concentration was found in *L. purpureus* subsp. *uncinatus*, followed by *C. argyraea*; the lowest P concentration was found in *C. heidmannii*. Samples from TAZAC had higher ( $P < 0.05$ ) P concentrations than those from other locations; samples from the road sides were similar ( $P > 0.05$ ) in P concentration to those from Neudamm. The ash concentration (%) was influenced ( $P < 0.05$ ) by the legume species and year of harvest. *R. totta* (Thunb.) DC. var. *totta* had the highest ash content ( $12.7 \pm 1.5$ ); the least ash was observed in *M. candicans* ( $7.7 \pm 1.5$ ). Samples harvested in 2014 had lower ( $P < 0.05$ ) ash content compared to those of 2015 ( $9.3 \pm 0.5$  vs.  $10.6 \pm 0.6$ , respectively).

The computed TDN, DE and ME for the legumes are presented in Table 3.3. TDN for the legumes varied from 57.4 to 70.6%. The TDN of *C. argyraea* was similar to that of *M. sativa*; *C. heidmannii* had the highest TDN (70.6%). The ME (MJ/kg) for the legumes was in the range 8.7 (*L. purpureus* subsp. *uncinatus* [wild type lablab]) to 10.7 (*C. heidmannii*). The average TDN and ME of the grasses used in the feeding trial were 66% and 10 MJ/kg, respectively.

The crude fiber (CF) concentration differed ( $P = 0.0012$ ) among the legumes. Results of the Kruskal-Wallis test are shown in Table 3.4. Pair-wise comparisons of legumes for CF was only done for pre-planned comparisons. Pairs of legumes that were significantly different together with the associated probabilities were: *C. argyraea* and *O. burchellii* ( $P = 0.0311$ ); *C. argyraea* and *V. lobatifolia* ( $P = 0.0356$ ); *V. lobatifolia* and *L. purpureus* subsp. *purpureus* ( $P = 0.0136$ ); *V. lobatifolia* and *C. podocarpa* ( $P = 0.0356$ ); *O. burchellii* and *L. purpureus* subsp.

*purpureus* ( $P = 0.0109$ ); and lucerne and *M. candidans* ( $P = 0.016$ ); lucerne and *C. argyrea* ( $P = 0.0396$ ); and lucerne and *C. podocarpa* ( $P = 0.0396$ ). A tendency was observed for the pairs: Lucerne and *L. purpureus* subsp. *purpureus* ( $P = 0.0855$ ); and *C. argyrea* and *L. purpureus* subsp. *purpureus* ( $P = 0.0756$ ). There were no differences ( $P > 0.05$ ) for the pairs: *C. argyrea* and *C. podocarpa*; *O. burchellii* and *V. lobatifolia*; lucerne and *V. lobatifolia*; and lucerne and *O. burchellii*.

**Table 3.1:** Univariate statistics of the chemical analyses of the forage legumes

Variable (%)*	n	Minimum	Maximum	Mean	s.d	C.V (%)
DM	60	88.97	96.14	93.723	1.160	1.2
CP	60	9.95	33.86	20.574	4.375	21.3
ADF	60	17.84	45.82	29.282	6.498	22.2
NDF	60	30.69	57.93	42.387	7.562	17.8
CF	58	13.0	40.50	24.042	7.534	31.3
Ash	60	6.54	19.11	10.238	2.855	27.9
EE	60	0.81	5.23	2.481	1.049	42.3
Ca	57	0.5	7.57	2.163	1.456	67.3
P	56	0.02	1.11	0.255	0.287	112.7
TDN	60	56.482	70.456	64.455	3.880	6.0
DE (MJ/kg)	60	10.398	12.971	11.866	0.714	6.0
ME (MJ/kg)	60	8.526	10.636	9.730	0.586	6.0

\* Units are % except where where indicated. s.d. = standard deviation; C.V = coefficient of variation (%)

**Table 3.2:** Least squares means ( $\pm$  S.E) of chemical composition (% DM) of forage legumes

Legume	CP	ADF	NDF	Ash	EE	Ca	P
<i>C. argyraea</i>	21.6 $\pm$ 2.5 <sup>b</sup>	23.4 $\pm$ 3.2 <sup>b</sup>	36.2 $\pm$ 3.8 <sup>a</sup>	12.0 $\pm$ 1.8 <sup>a</sup>	8.7 $\pm$ 0.6 <sup>a</sup>	1.4 $\pm$ 0.7 <sup>a</sup>	0.8 $\pm$ 0.1 <sup>d</sup>
<i>C. heidmannii</i>	14.6 $\pm$ 2.5 <sup>ac</sup>	16.3 $\pm$ 3.2 <sup>a</sup>	30.3 $\pm$ 3.8 <sup>a</sup>	10.1 $\pm$ 1.8 <sup>a</sup>	2.3 $\pm$ 0.6 <sup>c</sup>	0.8 $\pm$ 0.7 <sup>a</sup>	-0.2 $\pm$ 0.1 <sup>a</sup>
<i>C. podocarpa</i>	24.2 $\pm$ 2.5 <sup>b</sup>	20.0 $\pm$ 3.2 <sup>ab</sup>	44.3 $\pm$ 3.8 <sup>a</sup>	12.0 $\pm$ 1.8 <sup>a</sup>	5.3 $\pm$ 0.6 <sup>b</sup>	1.0 $\pm$ 0.7 <sup>a</sup>	0.2 $\pm$ 0.1 <sup>b</sup>
<i>C. tomentosum</i>	20.4 $\pm$ 2.0 <sup>ac</sup>	35.1 $\pm$ 2.5 <sup>de</sup>	51.3 $\pm$ 3.1 <sup>b</sup>	8.8 $\pm$ 1.4 <sup>a</sup>	1.9 $\pm$ 0.5 <sup>d</sup>	2.3 $\pm$ 0.6 <sup>b</sup>	0.1 $\pm$ 0.1 <sup>b</sup>
<i>L. purpureus</i> <sup>1</sup>	22.8 $\pm$ 2.1 <sup>b</sup>	19.5 $\pm$ 2.3 <sup>ab</sup>	39.2 $\pm$ 3.2 <sup>a</sup>	9.4 $\pm$ 1.5 <sup>a</sup>	1.6 $\pm$ 0.5 <sup>d</sup>	2.4 $\pm$ 0.6 <sup>b</sup>	0.2 $\pm$ 0.1 <sup>b</sup>
<i>L. purpureus</i> <sup>2</sup>	18.7 $\pm$ 2.1 <sup>ac</sup>	28.3 $\pm$ 2.3 <sup>bc</sup>	56.1 $\pm$ 3.2 <sup>b</sup>	9.7 $\pm$ 1.5 <sup>a</sup>	2.4 $\pm$ 0.5 <sup>c</sup>	2.0 $\pm$ 0.5 <sup>b</sup>	1.0 $\pm$ 0.1 <sup>d</sup>
<i>M. candicans</i>	12.3 $\pm$ 2.1 <sup>a</sup>	38.8 $\pm$ 2.7 <sup>e</sup>	52.7 $\pm$ 3.2 <sup>b</sup>	7.7 $\pm$ 1.5 <sup>ab</sup>	2.5 $\pm$ 0.5 <sup>c</sup>	1.2 $\pm$ 0.6 <sup>a</sup>	0.3 $\pm$ 0.1 <sup>c</sup>
<i>M. laciniata</i>	20.4 $\pm$ 2.5 <sup>a</sup>	24.1 $\pm$ 3.0 <sup>a</sup>	43.5 $\pm$ 3.8 <sup>ac</sup>	10.7 $\pm$ 1.8 <sup>a</sup>	2.7 $\pm$ 0.6 <sup>c</sup>	1.7 $\pm$ 0.7 <sup>b</sup>	.
<i>M. sativa</i>	22.7 $\pm$ 1.2 <sup>b</sup>	26.9 $\pm$ 1.5 <sup>b</sup>	36.0 $\pm$ 1.8 <sup>a</sup>	10.9 $\pm$ 0.8 <sup>a</sup>	1.6 $\pm$ 0.3 <sup>d</sup>	1.1 $\pm$ 0.3 <sup>a</sup>	0.2 $\pm$ 0.1 <sup>b</sup>
<i>O. burchellii</i>	22.2 $\pm$ 1.1 <sup>b</sup>	28.2 $\pm$ 1.6 <sup>bc</sup>	44.2 $\pm$ 1.7 <sup>ac</sup>	8.0 $\pm$ 0.8 <sup>ab</sup>	4.0 $\pm$ 0.3 <sup>b</sup>	2.5 $\pm$ 0.4 <sup>b</sup>	0.3 $\pm$ 0.1 <sup>c</sup>
<i>P. biflorum</i>	20.7 $\pm$ 1.6 <sup>a</sup>	21.6 $\pm$ 2.1 <sup>ab</sup>	34.1 $\pm$ 2.4 <sup>a</sup>	11.1 $\pm$ 1.1 <sup>a</sup>	2.9 $\pm$ 0.4 <sup>c</sup>	1.4 $\pm$ 0.5 <sup>a</sup>	0.1 $\pm$ 0.1 <sup>b</sup>
<i>R. fleckii</i>	19.9 $\pm$ 1.5 <sup>acd</sup>	32.0 $\pm$ 2.0 <sup>cd</sup>	45.3 $\pm$ 2.4 <sup>b</sup>	8.8 $\pm$ 1.1 <sup>a</sup>	2.9 $\pm$ 0.4 <sup>c</sup>	1.4 $\pm$ 0.5 <sup>a</sup>	0.1 $\pm$ 0.1 <sup>b</sup>
<i>R. totta</i> (Thunb.)	16.6 $\pm$ 2.1 <sup>ac</sup>	27.2 $\pm$ 2.7 <sup>b</sup>	44.7 $\pm$ 3.2 <sup>b</sup>	12.7 $\pm$ 1.5 <sup>a</sup>	3.7 $\pm$ 0.5 <sup>bc</sup>	4.2 $\pm$ 0.6 <sup>c</sup>	0.4 $\pm$ 0.1 <sup>c</sup>
<i>V. lobatifolia</i>	21.0 $\pm$ 1.2 <sup>b</sup>	27.6 $\pm$ 1.7 <sup>b</sup>	44.4 $\pm$ 1.9 <sup>ac</sup>	9.3 $\pm$ 0.9 <sup>a</sup>	2.6 $\pm$ 0.3 <sup>c</sup>	2.3 $\pm$ 0.4 <sup>b</sup>	0.2 $\pm$ 0.1 <sup>b</sup>
P-value	< 0.05	< 0.05	< 0.0001	< 0.05	< 0.05	< 0.05	< 0.05
CV (%)	8.1	11.8	12.5	19.0	29.1	48.3	41.1
R <sup>2</sup>	.59	.80	.63	.43	.78	.84	0.88

Least squares means with different superscripts within a column differ ( $P < 0.05$ ). <sup>1</sup>*L. purpureus* is the cultivated lablab (*Lablab purpureus*) subsp. *purpureus*; <sup>2</sup>*L. purpureus* is the unimproved lablab found in the wild, with full nomenclature given as: *Lablab purpureus* (L.) sweet subsp. *uncinatus* verde. var. *rhomboideus* (Schinz) verde.

**Table 3.3:** Least squares means ( $\pm$  S.E) of NDF, TDN and predicted energy content of forage legumes and grass hay<sup>3</sup>

Legume	NDF (%)	TDN (%)	DE (MJ/kg)	ME (MJ/Kg)
<i>C. argyraea</i>	36.2 $\pm$ 3.8 <sup>a</sup>	67.6 $\pm$ 2.0 <sup>bd</sup>	12.5 $\pm$ 0.4 <sup>b</sup>	10.2 $\pm$ 0.3 <sup>b</sup>
<i>C. heidmannii</i>	30.3 $\pm$ 3.8 <sup>a</sup>	70.6 $\pm$ 2.0 <sup>cd</sup>	13.0 $\pm$ 0.4 <sup>b</sup>	10.7 $\pm$ 0.3 <sup>bc</sup>
<i>C. podocarpa</i>	44.3 $\pm$ 3.8 <sup>a</sup>	63.5 $\pm$ 2.0 <sup>bd</sup>	11.7 $\pm$ 0.4 <sup>b</sup>	9.6 $\pm$ 0.3 <sup>b</sup>
<i>C. tomentosum</i>	51.3 $\pm$ 3.1 <sup>b</sup>	59.9 $\pm$ 1.6 <sup>a</sup>	11.0 $\pm$ 0.3 <sup>a</sup>	9.0 $\pm$ 0.2 <sup>a</sup>
<i>L. purpureus</i> <sup>1</sup>	39.2 $\pm$ 3.2 <sup>a</sup>	66.1 $\pm$ 1.6 <sup>bd</sup>	12.2 $\pm$ 0.3 <sup>b</sup>	10.0 $\pm$ 0.2 <sup>b</sup>
<i>L. purpureus</i> <sup>2</sup>	56.1 $\pm$ 3.2 <sup>b</sup>	57.4 $\pm$ 1.6 <sup>a</sup>	10.6 $\pm$ 0.3 <sup>a</sup>	8.7 $\pm$ 0.2 <sup>a</sup>
<i>M. candidans</i>	52.7 $\pm$ 3.2 <sup>b</sup>	59.1 $\pm$ 1.6 <sup>a</sup>	10.9 $\pm$ 0.3 <sup>a</sup>	8.9 $\pm$ 0.2 <sup>a</sup>
<i>M. laciniata</i>	43.5 $\pm$ 3.8 <sup>ac</sup>	63.9 $\pm$ 2.0 <sup>bd</sup>	11.8 $\pm$ 0.4 <sup>b</sup>	9.6 $\pm$ 0.3 <sup>b</sup>
<i>M. sativa</i>	36.0 $\pm$ 1.8 <sup>a</sup>	67.7 $\pm$ 0.9 <sup>bd</sup>	12.5 $\pm$ 0.2 <sup>b</sup>	10.2 $\pm$ 0.1 <sup>b</sup>
<i>O. burchellii</i>	44.2 $\pm$ 1.7 <sup>ac</sup>	63.5 $\pm$ 0.9 <sup>bd</sup>	11.7 $\pm$ 0.2 <sup>b</sup>	9.6 $\pm$ 0.1 <sup>b</sup>
<i>P. biflorum</i>	34.1 $\pm$ 2.4 <sup>a</sup>	68.7 $\pm$ 1.2 <sup>bd</sup>	12.6 $\pm$ 0.2 <sup>b</sup>	10.4 $\pm$ 0.2 <sup>b</sup>
<i>R. fleckii</i>	45.3 $\pm$ 2.4 <sup>b</sup>	62.9 $\pm$ 1.2 <sup>bd</sup>	11.6 $\pm$ 0.2 <sup>b</sup>	9.5 $\pm$ 0.2 <sup>b</sup>
<i>R. totta</i> (Thunb.)	44.7 $\pm$ 3.2 <sup>b</sup>	63.3 $\pm$ 1.6 <sup>bd</sup>	11.7 $\pm$ 0.3 <sup>b</sup>	9.6 $\pm$ 0.2 <sup>b</sup>
<i>V. lobatifolia</i>	44.4 $\pm$ 1.9 <sup>ac</sup>	63.4 $\pm$ 1.0 <sup>bd</sup>	11.7 $\pm$ 0.2 <sup>b</sup>	9.6 $\pm$ 0.1 <sup>b</sup>
Grass hay	59.1 $\pm$ 2.7 <sup>b</sup>	55.9 $\pm$ 1.4 <sup>a</sup>	10.3 $\pm$ 0.3 <sup>a</sup>	8.4 $\pm$ 0.2 <sup>a</sup>

Least squares means with different superscripts within a column differ ( $P < 0.05$ ). <sup>1</sup>*L. purpureus* is the cultivated lablab (*Lablab purpureus*) subsp. *purpureus*; <sup>2</sup>*L. purpureus* is the unimproved lablab found in the wild, with full nomenclature given as: *Lablab purpureus* (L.) sweet subsp. *uncinatus* verde. var. *rhomboideus* (Schinz) verde.

<sup>3</sup>TDN was computed using the equation: TDN (%) = 86.2 – (0.513 x %NDF) for legumes; and TDN (%) = 105.2 – (0.667 x %NDF) for grass hay.

It was assumed 1 kg TDN yields 4.4 MCal of digestible energy (DE) and ME is 82% of DE (NRC, 2007).

To convert MJ/kg to MCal/kg divide by 4.184 e.g. for *C. argyraea* ME = 10.2/4.184 = 2.4 MCal/kg.

**Table 3.4:** Wilcoxon scores (rank sums) used in the Krusk-Wallis non-parametric test for crude fibre (CF) concentration (% DM) of forage legumes<sup>3</sup>

Legume	n	Sum of scores	Mean score
<i>Crotalaria argyrea</i>	2	5.0	2.5
<i>Clotalaria heidmanni</i>	2	89.0	44.5
<i>Clotalaria podocarpa</i>	2	5.0	2.5
<i>Cullen tomentosum</i>	3	160.5	53.5
<i>Lablab purpureus</i> <sup>1</sup>	3	21.0	7.0
<i>Lablab purpureus</i> <sup>2</sup>	3	88.0	29.3
<i>Meulobium candicans</i>	3	145.0	48.3
<i>Medicago laciniata</i>	2	66.0	33.0
<i>Medicago sativa</i>	7	163.0	23.3
<i>Otoptera burchellii</i>	10	269.0	26.9
<i>Ptychlobium biflorum</i>	5	191.0	38.2
<i>Rhynchosia fleckii</i>	5	220.0	44.1
<i>Rhynchosia totta</i> (Thunb.)	3	64.0	21.3
<i>Vigna lobatifolia</i>	8	224.0	28.0

<sup>3</sup>Average scores were used for ties.

<sup>1</sup>*L. purpureus* is the cultivated lablab (*Lablab purpureus*) subsp. *purpureus*; <sup>2</sup>*L. purpureus* is the unimproved lablab found in the wild, with full nomenclature given as: *Lablab purpureus* (L.) sweet subsp. *uncinatus* verde. var. *rhomboideus* (Schinz) verde.

### 3.4 Discussion

The potential of indigenous forage legumes as feed for ruminants was the primary focus of this investigation. The hypothesis (i) of equality of chemical composition for all legumes was rejected. The legumes belong to different species and genera; they were also harvested from different locations (differing in soils and other climatic variables) and in different growing seasons, which is reflected by the year effect. The minimum dietary CP concentration for various classes of dairy cattle consuming leafy pasture is 134-168 g/kg DM (Holmes et al., 2007), which implies that with the exception of *M. candidans*, all the legumes have potential as livestock feed. The CP (% DM) of *L. purpureus* subsp. *purpureus* is similar to reported values of 10 – 22% for the whole plant (Adebisi and Bosch, 2004). The leaves of *L. purpureus* also do not contain tannins (Adebisi and Bosch, 2004). Alfalfa had a CP of  $20.3 \pm 0.1$  (Kanani et al., 2006), which is similar to what was obtained in this study.

In a study with Boer x Spanish goat crosses on a 70% concentrate diets, CP of 14% was satisfactory with seemingly no benefit from addition of RUDP (Prieto et al., 2000). Thus the CP levels for all legumes except *M. candidans* would appear satisfactory for fast growing meat goats such as the Boer goats used in this study. Other chemical constituents (ADF, NDF, ash) for Lucerne were similar to those reported in other studies (Kanani et al., 2006; Criscioni et al., 2016).

The CP of the indigenous forage legumes *C. argyrea*, *C. podocarpa*, *O. burchellii* and *V. lobatifolia* was similar ( $P > 0.05$ ) to that of the improved forage legumes *L. purpureus* and *M. sativa*. This is surprising given that improved forage legumes have been genetically selected for high performance in a number of attributes including nutritive value. Moreover, the indigenous forage legumes were collected from the wild, growing in most cases under suboptimal

conditions. Also, the potential exists that varieties of the forage legumes exist in the wild which could expand the range of nutritive values for many of the parameters in the chemical analyses. The wild and cultivated lablab differed ( $P < 0.05$ ) in CP content which reflects the improvement in nutritive value of *L. purpureus* subsp. *purpureus* as a result of genetic selection.

On account of the higher ADF and NDF concentrations, it is expected that *C. tomentosum*, *M. candicans*, *R. fleckii* and *R. totta* (Thunb.) DC. var *totta* and wild lablab should have lower digestibility than other legumes (Ellis et al., 2005; Foster et al., 2009). The NDF (% DM) varied from  $30.3 \pm 3.8$  (*C. heidmannii*) to  $56.1 \pm 3.2$  (*L. purpureus* subsp. *uncinatus*). Van Soest (1994) suggested that NDF concentration lower than 550-600 g/kg DM would not limit intake, hence all the forage legumes evaluated in this study were within an acceptable range for potentially high intake. Notwithstanding, Cannas (2002) recommended a maximum of 38% NDF in diets for lactating ewes, hence legumes with high NDF (*C. tomentosum*, *M. candicans*, *L. purpureus* subsp. *uncinatus*) may lead to diets exceeding this threshold, which may adversely affect intake and milk yield or other performance measures.

In studies with barley and whole crop wheat forages, Weisbjerg et al. (2003) reported that both NDF and ADF, but not acid detergent lignin (ADL) were significantly and negatively correlated with the rate of NDF digestion. Similarly, Wilman et al. (1996) found that the rate of cell wall digestion was negatively correlated ( $r = -0.81$ ) with silage NDF and suggested that leafier crops had a more rapid rate of digestion. The inverse relationship between NDF concentration and NDF digestion rate may be due to thickened cell walls which are resistant to particle breakdown and microbial penetration (Mertens, 1993a). Based on the ADF, NDF and degree of leafiness, the forage legumes *C. argyraea*, *C. heidmannii*, *O. burchellii*, *V. lobatifolia* and *R. fleckii* may have rapid digestion, leading to faster rates of passage hence permitting higher

voluntary intakes, compared to other native legumes. The rates of digestion for these forage legumes may indeed be comparable to lucerne and improved lablab, given the similarity in ADF and NDF concentration.

The digestibility of the NDF fraction is affected by the chemical composition of the NDF (proportions of cellulose, hemicelluloses and lignin). The implication is that diets or feeds with similar NDF concentration will not necessarily have similar net energy of lactation (NE<sub>L</sub>) concentration and certain feeds or diets with high NDF may have more NE<sub>L</sub> than another feed or diet with lower concentrations of NDF (NRC, 2001).

The indigestibility of the cell wall is mainly due to lignin. In temperate forages within a given plant growth cycle, the Weende crude fiber values increase in proportion to the cell wall content (Aufrere and Guerin, 1996). Hence crude fibre is indicative of cell wall indigestibility for a given plant. Plants with the same crude fibre can, however, differ in digestibilities depending on plant species and growth cycle. The ranking of the worst three legumes based on crude fibre values were: *M. candicans* > *C. tomentosum* > *L. purpureus* subsp. *uncinatus*, which correspond to the high observed ADF and NDF concentrations relative to other legumes, which signals poor digestibility. The shrub *M. candicans* develops thorns as it matures and this may contribute to the high CF value. *C. tomentosum* was observed to be frost resistant and usually survives mild winters (Kaholongu, 2016) and it is plausible plants accumulate high lignin content as they mature over several seasons of growth contributing to the high CF values. Wild lablab was peculiar in having moderate CF, despite having the highest NDF (56.2 ± 3.1%). The expected lower digestibilities of *C. tomentosum* and *M. candicans* would suggest low nutritive value of these forage legumes, except during early vegetative stage.



An approximation to the fatty acid (FA) content of a feed is  $FA = EE - 1$  (Allen, 2000). The FA observed for *M. sativa* (0.6) is lower than that reported for *M. sativa* (1.6 – 1.72%) (Boufaied et al., 2003). The fatty acid composition of a given feed ingredient is required; specifically in ruminants a high proportion of unsaturated fatty acids will depress fibre digestion in the rumen (McDonald et al., 2010). The NRC (2001) recommends total dietary fat not to exceed 6 – 7% of dietary DM, hence it would seem that because of the high FA concentration (7.7%) in *C. argyraea*, only moderate levels should be used in diets because of possible reduction in DM intake, even though the fat may have negligible effects on ruminal fermentation (Schauff and Clark, 1992). The advantage of using fat to increase energy density will be partly or fully negated by reduced DM intake, which can limit milk production responses (NRC, 2001). The high levels of EE found in *C. argyraea* may also lead to rancidity during storage of this legume and appreciably affect the nutritive value of this forage legume hay under long term storage.

Key factors influencing the fat and fatty acid composition of forages are species (and to a less extent cultivar), vegetation stage, conditions of conservation and N fertilization (Dewhurst et al., 2006; Khan et al., 2012). Meta-analyses indicated that vegetation stage is the predominant factor (Khan et al., 2012; Glasser et al., 2013); with increasing maturity of forage there is a decrease in proportion of leaves (Dewhurst et al., 2001) which are richer than stems and seeds in membrane lipids and subsequently senescence of leaves and an increase in the fiber and storage fractions (grain). Poor drying conditions during hay making could result in lipolysis and oxidation of the poly unsaturated fatty acids (Molloy et al., 1975; Dewhurst et al., 2006); there could also be loss of leaves during forage handling or extended respiration within plant cells (Baumont et al., 2011). In this study, a combination of species and vegetation stage at harvesting

may have influenced the estimated FA because harvests were done when the plants were at different stages of maturity depending on when active regrowth started after the cold winter period (June to September).

The mineral content of pastures varies a lot and depends on the species, stage of growth, soil type, cultivation conditions and fertilizer application (McDonald et al., 2010). When legumes and grasses are cultivated under same soil types and with same fertilizer treatment, legumes generally contain higher mineral concentrations particularly for calcium, magnesium, copper and cobalt (Holmes et al., 2007). The minerals that are most likely to be deficient in feed for all food animals are calcium and phosphorous (MacDonald et al., 2010) and hence were considered in the analysis.

About 20 to 30% of calcium within in plants is bound to oxalate which is relatively unavailable to the ruminant (Ward et al., 1979). Because alfalfa is a major contributor of calcium in dairy rations, absorption of calcium from alfalfa is used as an estimate of efficiency of absorption of calcium from forages in general. An efficiency of absorption of 30% is used in the model for calcium from forages (NRC, 2001). Using this efficiency of absorption, the maximum amount of calcium is from *R. totta* (Thunb.) DC. var. *totta* at 1.26% (equivalent to 12.6 g/kg DM) which would be sufficient to support requirements for heifers and cows (Holmes et al., 2007). The legumes *R. fleckii*, *C. Heidmannii*, *C. podocarpa*, *M. candicans* and *M. sativa* would not be able to meet requirements for calcium for heifers and cows from pasture alone and would need to be supplemented from other sources. The calcium requirements for 20 kg live weight growing lambs with a dry matter intake of 0.46kg/day of metabolisability ( $q_m$ ) of 0.55, with a live weight gain of 150g/day would be 0.74% of diet DM (McDonald et al., 2010). Among the native legumes, only *R. totta* (Thunb.) DC. var. *totta* and *O. burchellii* would be able to meet

these requirements and supplemental sources of calcium would be needed for lambs consuming other forage legumes.

The phosphorous requirements for lactating dairy cows with a pasture intake of 17.4 kg DM/day is 0.375% of diet DM (McDonald et al., 2010). Taking into account availability of 30 – 70% for phosphorous (McDonald et al., 2010), most of the legume hays would not be able to supply the requirements for dairy cattle. The forage legumes would similarly be unable to meet phosphorous requirements for 20 kg growing lambs with a dry matter intake of 0.46kg/day of metabolisability ( $q_m$ ) of 0.55, with a live weight gain of 150g/day, estimated at 0.65% (McDonald et al., 2010).

In the past the calcium to phosphorous ratio was thought to be important in absorption of calcium and phosphorous, but recent data suggest that the calcium: phosphorous ratio is not critical unless the ratio  $> 7:1$  or  $< 1:1$  (NRC, 2001). The legumes with a more desirable balance of Ca: P are *C. argyrea*, *C. podocarpa*, *M. candicans*, Lucerne and *L. purpureus* subsp *purpureus*. Imbalances of Ca to P (Ca: P  $> 7$  but not exceeding 15) were observed in *O. burchellii*, *R. fleckii*, *R. totta* (Thunb.) DC. var. *totta* and *V. lobatifolia*. The worst imbalance (23:1) for the two minerals was in *C. tomentosum*, which would need to be supplemented with phosphorous to achieve better balance. As pastures mature, concentrations of most minerals decrease, however, season effects may also be important. For example in New Zealand concentrations of Ca and Mg are higher and P lower in summer than spring (Holmes et al., 2007). Future studies could examine the impact of season on mineral concentrations of forage legumes.

Assessment of feeding value of forages is done using dietary ME; ME is also used to ration dairy cattle (Waghorn and Clark, 2004; Yan and Agnew, 2004). The ME of the legume

forages lucerne, *L. purpureus* subsp. *purpureus*, *C. argyraea* and *P. biflorum* is moderate and similar. *L. purpureus* subsp. *uncinatus* and *C. candicans* had the lowest ME on account of their high NDF concentration. High quality forages have >10.5 MJ ME/kg DM (Holmes et al., 2007) and hence only *C. heidmannii* would fall into this category. For dairy cattle with an assumed maintenance requirement of 54 MJ/day (McDonald et al., 2010) together with intake restrictions due to fill effects, there would be need to supplement with energy concentrates. The metabolisable energy requirements for growing 20 kg lambs with an intake of 0.56 kg DM/day of a diet with metabolisability ( $q_m$ ) of 0.65, gaining 150 g/day is 6.5 MJ ME/day (McDonald et al., 2010). All the forage legumes potentially can meet this requirement, but palatability and digestibility may be limiting factors.

The chemical composition of *C. argyraea* and *P. biflorum* was similar to that of *M. sativa* and *L. purpureus* subsp. *purpureus* in a number of aspects (CP, ADF and NDF). Notwithstanding, the nutritive value will be influenced by intake levels, the digestibility and presence of antinutrients and their concentration in the feed resource. The *Crotalaria* species are known to contain pyrrolizidine alkaloids (Lucena et al., 2010) which cause toxicity in livestock. Pyrrolizidine and other alkaloids are metabolized to more toxic compounds within the animal (Cheeke, 1998; Acamovic et al., 2004). The forage legumes *M. laciniata*, *O. burchellii* and *V. lobatifolia* have CP and NDF concentrations similar to those of *L. purpureus* subsp. *purpureus*. The morphological structure of *M. laciniata* (has tiny leaves and forms a mat on the ground) renders it difficult to graze, except possibly by sheep. Further studies are needed to shed light on the nutritive value of the forage legumes with desirable chemical composition and how they could be exploited in grazing systems or under cutting.

The chemical composition (CP, NDF, ADF, ash) of *L. purpureus* subsp. *purpureus* is similar to the results reported by Kanani et al. (2006). Makembe et al. (1996) reported, however, much lower level of CP (13.5 %) and much higher level of ADF (46.6%) for *L. purpureus* subsp. *purpureus*, which may be attributed to differences in soils where the lablab was grown. Wild lablab (*L. purpureus* subsp. *uncinatus*) had a higher ( $P < 0.05$ ) level of ADF and NDF compared to *L. purpureus* subsp. *purpureus*, implying its digestibility will be lower; wild lablab also has proportionately much smaller leaves, but may have other attributes of importance in genetic selection. The results show that wild lab compares poorly to *L. purpureus* subsp. *purpureus* in a number of respects: CP, ADF, EE and phosphorus, which could be attributed to genetic improvement through selection for the latter subspecies. The chemical composition of Lucerne was similar to the reported values by Ngwa et al. (2003) except for NDF and ADF which were reported to be higher at 40.2 and 34.1, respectively.

Some of the differences in chemical composition were influenced by location of harvest and year when the samples were collected. Location differences may reflect soil and/or climatic differences that affect chemical composition. Chemical properties of the soil for instance pH may determine the nutrients that can be absorbed by plants. Phosphorous for example is most available when soil pH is between 6 and 7. In soils of low pH, P reacts with hydrous oxides of iron, aluminum and magnesium to form insoluble compounds which are unavailable to plants. At  $\text{pH} \geq 7$ , P becomes insoluble as calcium phosphate (Oelberg, 1956). While the P content of oats paralleled the total P content of the soil, that of red clover paralleled that of available P content of the soil (Holtz, 1930). Year differences arose due to different months in which the samples were obtained which would influence the phenological stage (early bud, late bloom) when they were

sampled; but even for samples collected in the same month, differences could arise across years due to the time when the rain season commenced.

The differences in chemical composition underscores the importance of maintaining diversity in composition of pastures for grazing ruminants because the different legumes complement each other in meeting the animal's needs for the different nutrients. Furthermore, selective grazing further enables the animal to obtain its nutrient requirements and also to dilute any toxic chemicals (Freeland and Janzen, 1974). Differences in chemical composition will influence proteolysis (for instance as indicated by the concentration of free amino acids and ammonia) during ensiling of forage legumes (Albrecht and Muck, 1991; Mustafa and Seguin, 2003; Contreras-Govea et al., 2009).

The nutrient content of forage legumes can be influenced by many factors (stage of growth, fertilizer application, soils, season); nonetheless, equations can be developed that predict nutrient digestibility and energy concentration based on the chemical composition (Stergiadis et al., 2015). This enables rapid screening of forage legumes for nutritive quality using chemical composition data that may be readily obtained. Plant growth habits and morphological characteristics complements the chemical analysis data to rapidly screen forage legumes to manageable numbers that can undergo more detailed studies for example agronomic characterization, *in vivo* digestibility, palatability, voluntary intake and impact on animal performance (growth rate, milk production and carcass quality) before recommendations can be made.

On these bases of favourable chemical composition and plant morphology, the legumes *C. argyrea*, *C. heidmannii*, *C. tomentosum*, *L. purpureus* subsp. *purpureus*, *L. purpureus* subsp. *uncinatus*, *O. burchellii*, *P. biflorum*, *R. fleckii*, *R. totta* (Thunb.) DC. var. *totta* and *V. lobatifolia*

warrant further studies. Further studies including assays for antinutrients may shrink this list and make recommendations on potential use form (grazed *in situ* and/or fodder production). It should be borne in mind that chemical analyses provide only some indication of utility of feeds or diets; differences in the chemical composition of NDF for example, could result in different digestibilities of DM, OM and NDF for feeds or diets with similar NDF concentration (NRC, 2001).

### **3.5 Conclusions**

All legumes, except *M. candicans*, exceeded the minimum threshold of 13% CP for feeding dairy cattle (Holmes et al., 2007) and had NDF concentration well below the 550-600 g/kg DM that would limit intake (Van Soest, 1994), hence they have potential to contribute to the diversity of feed resources that can sustain ruminant livestock productivity in harsh semi-arid environments like those in central Namibia. The results of chemical analyses of feeds are useful in general assessments of the potential value of feeds; nonetheless, the true value of individual constituents can only be obtained by measuring the extent to which the ruminant animal utilises them (Holmes et al., 2007). These legumes, therefore need to be tested for digestibility, palatability, assayed for antinutrients, voluntary feed intake and their agronomic aspects need to be thoroughly investigated.

## CHAPTER 4

### 4 Assessment of relative palatability of five (5) selected forage legumes fed to weaned Boer goats [Objective (ii)]

#### 4.1 Introduction

Palatability is defined as plant characteristics or conditions which stimulate a selective response by animals (Todaro et al., 2007). Palatability of forage is affected by both plant and animal factors such as growth stage, degree of maturity, chemical composition, morphology and phenology of the plant and differential preference for forage species by a particular animal, physiological status, general health and hunger of the animal (Amjad et al., 2014; Baumont, 1996). Concentration and/or availability of elements in forages may influence their digestibility, intake and palatability (Minson, 1990; Reid and Jung, 1991). Proximate analyses of the indigenous forage legumes which was part of this study, indicated they are high in crude protein and have acceptable concentration of NDF and ADF, which are predictors of intake and digestibility, respectively. There is, however, no comparative information on the palatability of the indigenous forage legumes *Otoptera burchellii*, *Crotalaria argyraea*, *Clotalaria podocarpa* and *Vigna lobatifolia*. The objective of this study was to evaluate preference of the four forage legumes relative to *Medicago sativa* as a standard. It was also of interest to empirically determine the length of the adaptation period of the weaned Boer goats to these 4 legumes.

#### 4.2 Materials and Methods

This experiment was conducted at the University of Namibia's Neudamm Campus to assess the relative feed preference of weaned Boer goats for four indigenous forage legume species viz. *O. burchellii*, *C. argyraea*, *C. podocarpa* and *V. lobatifolia* with *M. sativa*



(Lucerne/alfalfa) as a reference, through intake of hay by cafeteria method. A fifth legume *Cullen tomentosum* that was to be included as part of the palatability trial was eliminated because it was observed prior to commencement of the trial that goats rejected it completely possibly because of its strong odour. The four legumes (*O. burchellii*, *C. argyraea*, *C. podocarpa* and *V. lobatifolia*) were harvested from the wild during the growing season in 2014 and air-dried under a roofed house for a period of three weeks. The forage legumes *O. burchellii*, *C. argyraea* and *C. podocarpa* had woody stems and were trampled on a cement floor to remove the thick stems which were discarded. The material for the four legumes was packed in plastic bags and stored away from light until the feeding trial. The Lucerne hay was procured from a commercial retailer.

The eight (4 males and 4 females) weaned Boer goats weighing  $24.0 \pm 5.4$  kg were dewormed using IVOMEC<sup>®</sup> (Merial, South Africa) prior to the start of the experiment and were confined in a roofed and half-walled shed, with random allocation by sex to individual pens. A completely randomized design was used with each goat simultaneously offered 100 g of *O. burchellii*, *C. argyraea*, *C. podocarpa* and *V. lobatifolia* and 600 g of *M. sativa*. Each day the 100 g of *O. burchellii*, *C. argyraea*, *C. podocarpa* and *V. lobatifolia* and 600 g of *M. sativa* was weighed and placed in a random order in five (5) metallic feed containers within a pen. Grass hay, fresh water and mineralized salt licks were available at all times. Feeds were offered at 08h00 every morning for a total period of ten (10) days, of which 5 days was an adaptation period to allow the animals to adjust to both feed and confinement environment and five (5) days were the collection period. Before each feeding session, refusals were separately collected, weighed and stored for determination of dry matter.

Duplicate samples were obtained for each legume left over by each goat and dried in a furnace at 105°C for 24 h to determine DM. Percentage of legume consumed of that offered (DM basis) was computed and indicates relative palatability. Relative palatability computed this way is a percentage and has a binomial distribution, hence it was transformed by arcsine transformation to get an approximate normal distribution. After transformation and analysis, the resulting least square means were back-transformed and an error term for the original units was estimated using the untransformed data. Data was analysed by Proc Mixed (SAS, 2008) which takes into account correlation between repeated measures on an individual and the antecedent first order [ANTE(1)] covariance structure was assumed. Estimate statements were used in Proc Mixed (SAS, 2008) to compare means and obtain standard errors. Effects were considered significant at  $P < 0.05$ . Trends were declared when  $0.05 < P < 0.10$ .

### 4.3 Results

The univariate statistics for the variable relative palatability were: sample size ( $n = 387$ ); mean (82.2%); coefficient of variation ( $CV = 21.4\%$ ); standard error of mean (S. E. M = 0.89). Percentage of legume consumed of that offered was affected ( $P < 0.0001$ ) by legume species, day and legume x day interaction. The best covariance structure according to the Bayesian Information Criterion (BIC) was the antecedent order 1 [ANTE(1)]. The variances for days 1 to 10 are 626.0, 622.2, 28.9, 257.1, 16.0, 5.9, 6.0, 1.8, 4.3 and 2.6, respectively. There is evidence that variances in days 1 to 4 are very different from the remaining days, for instance the variance on day 4 is 16 times the variance on day 5.

Table 4.1 shows the least squares means of the percentage of legume consumed and how it varied by day over the trial period. There was wide fluctuation in the percentage consumed for *C. argyrae* and *C. podocarpa* in the first 4 days. Percentage of *C. argyrae* fluctuated from day 1

to about day 5; *C. podocarpa* consumed steadily rose from day 1 and stabilized at day 4. Nearly all the amounts offered of *O. burchellii*, *V. lobatifolia* and *M. sativa* were consumed from day 1 to day 10. In general, standard errors are large on the least square means for days 1 to 5, which is the adjustment period.

**Table 4.1:** Least squares means ( $\pm$  S.E) of percentage of legume consumed<sup>1, 2</sup>

Legume					
Day	CA	CPD	LC	OB	VL
1	64.0 $\pm$ 9.9 <sup>a</sup>	58.20 $\pm$ 9.9 <sup>a</sup>	99.9 $\pm$ 9.9	97.9 $\pm$ 10.6	98.6 $\pm$ 9.9
2	19.6 $\pm$ 10.0 <sup>b</sup>	56.0 $\pm$ 10.0 <sup>a</sup>	99.7 $\pm$ 10.7	98.2 $\pm$ 10.0	99.5 $\pm$ 10.0
3	95.8 $\pm$ 2.3 <sup>c</sup>	90.8 $\pm$ 2.3 <sup>b</sup>	100.0 $\pm$ 2.3	99.5 $\pm$ 2.3	100.0 $\pm$ 2.3
4	67.7 $\pm$ 6.9 <sup>a</sup>	94.9 $\pm$ 6.5 <sup>bd</sup>	99.8 $\pm$ 6.5	99.0 $\pm$ 6.5	100.0 $\pm$ 6.5
5	95.8 $\pm$ 2.1 <sup>c</sup>	94.9 $\pm$ 2.1 <sup>bd</sup>	99.8 $\pm$ 2.3	99.2 $\pm$ 2.3	99.7 $\pm$ 2.1
6	99.5 $\pm$ 0.5 <sup>c</sup>	99.0 $\pm$ 0.5 <sup>cd</sup>	100.0 $\pm$ 0.5	100.0 $\pm$ 0.5	100.0 $\pm$ 0.5
7	99.8 $\pm$ 0.3 <sup>c</sup>	99.7 $\pm$ 0.3 <sup>cd</sup>	100.0 $\pm$ 0.3	99.8 $\pm$ 0.3	100.0 $\pm$ 0.3
8	99.8 $\pm$ 0.1 <sup>c</sup>	99.7 $\pm$ 0.1 <sup>cd</sup>	100.0 $\pm$ 0.1	100.0 $\pm$ 0.1	100.0 $\pm$ 0.1
9	99.5 $\pm$ 0.2 <sup>c</sup>	99.8 $\pm$ 0.2 <sup>cd</sup>	100.0 $\pm$ 0.2	100.0 $\pm$ 0.2	99.9 $\pm$ 0.2
10	99.6 $\pm$ 0.3 <sup>c</sup>	99.9 $\pm$ 0.3 <sup>cd</sup>	100.0 $\pm$ 0.3	100.0 $\pm$ 0.4	100.0 $\pm$ 0.3

<sup>1</sup>CA = *C. argyraea*; CPD = *C. podocarpa*; LC = *M. sativa*; OB = *O. burchellii*; VL = *V. lobatifolia*.

<sup>2</sup>Least squares means within a column with different subscripts are different ( $P < 0.05$ )

We sought to identify the cause of the interaction by examining the differences of means for each day and the average of means for subsequent days for each legume (Table 4.2). These are labeled Day.1 to Day.9. For example Day.1 is interpreted as the difference of mean consumption of a legume and the average of the means for the legume from days 2 to Day 10. The first day that is not significantly different from the mean for subsequent days may be

considered the day that the response starts attaining a plateau (Littell et al., 1998). Maximum consumption of *C. argyraea* appears to start peaking at day 3 ([Day.3],  $P = 0.2163$ ); consumption of *C. podocarpa* on the other hand appears to start peaking at day 4 ([Day.4],  $P = 0.1513$ ). Conversely, goats consumed *M. sativa*, *V. lobatifolia* and *O. burchellii* at high levels from the first day right to day 10 (Day.1), with barely any refusals.

**Table 4.2:** Significance probabilities for tests on whether mean for each day differs from mean for subsequent days for each legume<sup>1</sup>

Legume	Day.1	Day.2	Day.3	Day.4	Day.5	Day.6	Day.7	Day.8	Day.9
CA	0.0150	0.0001	0.2163	0.0001	0.0001	0.5268	0.4236	0.0372	0.7729
CPD	0.0010	0.0002	<.0001	0.1513	<.0001	0.0009	0.2674	0.1903	0.5586
LC	0.9629	0.8381	0.9005	0.7703	0.2397	0.9078	0.8084	0.4101	0.4008
OB	0.5731	0.5641	0.4181	0.4904	0.0048	0.3246	0.0420	0.4346	0.4685
VL	0.5276	0.7362	0.6313	0.8307	0.1163	0.8012	0.4616	0.0993	0.0615

<sup>1</sup>CA = *C. argyraea*; CPD= *C. podocarpa*; LC = *M. sativa*; OB = *O. burchellii*; VL = *V. lobatifolia*.

It was of interest to compare the consumption of the legumes relative to *M. sativa* which is highly palatable and widely used in Namibia (hence a standard) to other legumes. Table 4.3 shows the estimates of differences between means for pre-planned contrasts for pairs of legumes after the 5 day adjustment period. Goats consumed significantly ( $P < 0.05$ ) more *M. sativa* than *C. argyraea*. More or less similar results were obtained when *M. sativa* was compared to *P. podocarpa*, except there was a trend ( $P = 0.0628$ ) for more *M. sativa* to be consumed than *C. podocarpa* on day 9; no difference in percentage consumed between *M. sativa* and *C. podocarpa* was evident ( $P > 0.1$ ) at day 10.

**Table 4.3:** Estimates of differences ( $\pm$  S.E) between means of legumes consumed over the 5 day test period<sup>1,2</sup>

<b>Contrast</b>	<b>Day</b>	<b>estimate</b>	<b>S.E</b>	<b>P-value</b>
CA – LC	5	-9.351	2.061	<.0001
CA – LC	6	-3.080	1.213	0.0116
CA – LC	7	-2.243	1.226	0.0684
CA – LC	8	-2.250	0.683	0.0011
CA – LC	9	-3.503	1.039	0.0008
CA – LC	10	-2.431	0.811	0.0029
CA – LC	Avg over days 5 – 10	-2.552	0.482	< 0.0001
CA – OB	Avg over days 5 – 10	-2.585	0.490	< 0.0001
CA – VL	Avg over days 5 – 10	-2.714	0.488	< 0.0001
LC – VL	5	0.559	2.061	0.7866
LC – VL	6	0.203	1.213	0.8675
LC – VL	7	-0.274	1.268	0.8288
LC – VL	8	-0.125	0.685	0.8554
LC – VL	9	1.641	1.039	0.1152
LC – VL	10	-0.935	0.811	0.2496
LC – VL	Avg over days 5 – 10	-0.162	0.484	0.7395
CA – CPD	5	1.194	2.000	0.5511
CA – CPD	6	1.838	1.213	0.1307
CA – CPD	7	0.584	1.226	0.6343
CA – CPD	8	0.175	0.700	0.8022
CA – CPD	9	-1.563	1.039	0.1336
CA – CPD	10	-1.844	0.811	0.0236
CA – CPD	Avg over days 5 – 10	-0.471	0.486	0.3390
CPD – LC	5	-10.545	2.061	<.0001
CPD – LC	6	-4.918	1.213	<.0001
CPD – LC	7	-2.826	1.226	0.0218
CPD – LC	8	-2.425	0.683	0.0004
CPD – LC	9	-1.940	1.039	0.0628
CPD – LC	10	-0.588	0.811	0.4691
CPD – LC	Avg over days 5 – 10	-2.080	0.482	0.0001
LC – OB	5	2.771	2.128	0.1938
LC – OB	6	-0.566	1.255	0.6525
LC – OB	7	2.077	1.264	0.1015

LC – OB	8	0.555	0.666	0.4051
LC – OB	9	0.447	1.068	0.6758
LC – OB	10	-1.080	0.839	0.1990
LC – OB	Avg over days 5 – 10	-0.034	0.486	0.9451
VL – OB	5	-2.213	2.069	0.2856
VL – OB	6	0.768	1.255	0.5409
VL – OB	7	-2.352	1.305	0.0725
VL – OB	8	-0.680	0.685	0.3214
VL – OB	9	1.194	1.068	0.2645
VL – OB	Avg over days 5 – 9	0.128	0.491	0.7953

<sup>1</sup>CA = *C. argyraea*; CPD = *C. podocarpa*; LC = *M. sativa*; OB = *O. burchellii*; VL = *V. lobatifolia*.

<sup>2</sup>Estimates of differences between means in degrees.

There were no significant differences ( $P > 0.05$ ) in proportions consumed when *M. sativa* was compared to *V. lobatifolia* and *O. burchellii*. There were no significant differences in percentage consumed for *C. argyraea* and *C. podocarpa* except on day 10 when goats consumed more *C. podocarpa* ( $P = 0.0236$ ). There were no differences ( $P > 0.05$ ) in percentages of *O. burchellii* and *V. lobatifolia* consumed. Also shown in Table 4.3 are the estimated differences between pairs of legumes, averaged over the days 5 to 10. There were no differences ( $P > 0.05$ ) between proportions consumed of *C. argyraea* and *C. podocarpa*; whereas the two *Crotalaria* species were consumed in lower ( $P = 0.0001$ ) proportions compared to Lucerne, *O. burchellii* and *V. lobatifolia*.

#### 4.4 Discussion

The hypothesis in this study was that there were no differences in the relative palatability of the different legumes fed to the weaned Boer goats. Striking differences are evident in legume consumption during the first 5 days of the trial: whereas nearly all of the *O. burchellii*, *V. lobatifolia* and *M. sativa* offered was consumed from day 1 up to day 10, there were wide

fluctuations in the proportions consumed for *C. argyraea* and *C. podocarpa*. In feeding trials it is customary to have an adjustment period which allows animals to adjust to the feed characteristics and also to pens. The large standard errors on the percentages consumed during the first four days, provide evidence of the large individual variability in feed intake and justifies having an adjustment period. Examination of the intake patterns shows there were wide fluctuations in individual consumption particularly of *C. argyraea* and *C. podocarpa* in the first four days. The the reported results from this experiment are therefore from days 5 to 10. Similarly, Ben Salem et al. (1994) and Kaitho et al. (1996) reported that feeding periods of less than 5 days gave wide variation in palatability results, whereas 5 to 12 days gave reliable results with R-square of up to 0.99. It is thought such fluctuations are a necessary part of the exploration of the nutritional environment of animals as it enables them to discover whether they can improve the nutritional balance from the currently selected proportions of foods (Forbes and Provenza, 2000).

Consumption of the *V. lobatifolia*, *O. burchellii* and *M. sativa* were essentially similar from day 1 to day 10 which may be explained partly by the high nutritive value and familiarity of the goats with these species. The chemical analyses for *V. lobatifolia* and *O. burchellii* done in experiment 1 indicated both forages had CP in excess of 14% CP; ADF and NDF values are within the acceptable ranges (Table 3.2). There may be low levels of antinutrients for *V. lobatifolia* and *O. burchellii* at the levels they were included in the diet or alternatively the goats have evolved mechanisms to combat their adverse effects, given their previous exposure to these forage legumes.

Although consumption patterns more or less reached a steady level of over 95% from day 5 to day 10, indicating that all the 5 legumes were quite palatable following an adjustment period, the *Clotalaria* species were consumed in lesser proportions. Examining the general trends

in days 5 to 10 indicates consumption of *C. argyrea* and *C. podocarpa* were similar and lower ( $P \leq 0.0001$ ) than Lucerne, *O. burchellii* and *V. lobatifolia*. The ADF and NDF of *C. argyrea*, *C. podocarpa* and *M. sativa* were similar ( $P > 0.05$ ); *M. sativa* had a lower ( $P < 0.05$ ) ADF and NDF than *O. burchellii* and *V. lobatifolia* (Table 3.2). The crude fibre (CF) concentration of *M. sativa* was higher ( $P = 0.0396$ ) than for *C. argyrea* and *C. podocarpa* (Table 3.4). Several aspects of the nutrient status of plants affect palatability including water soluble carbohydrate (WSC) concentration, fibre and nitrate (Jones and Roberts, 1991; Mayland et al., 2000; Horadagoda et al., 2009). Specifically WSC or sugars concentration is considered one of the key determinants of palatability. Dairy cows for example prefer sweet taste to all other tastes (Nombekela et al., 1994). NDF is an indicator of the lignin, cellulose and hemicellulose content of forages (Horadagoda et al., 2009). Although NDF is necessary to maintain normal rumen function (Van Soest et al., 1994; Beauchemin and Rode, 1997), cows prefer forages that are easier to graze and it is expected in general that animals prefer plants with lower NDF. The similarity in ADF and NDF concentration of *C. argyrea* and *C. podocarpa* to *M. sativa* (which is further re-enforced by the lower CF in the *Crotalaria* species relative to *M. sativa*) implies that fibre concentration was not an important determinant of differences in palatability in the present study.

Notwithstanding the lower NDF and ADF content of the *Crotalaria* species, weaned goats had higher initial consumption of *O. burchellii* and *V. lobatifolia* which may be the result of earlier exposures of the weaned goats to these legumes in the camps or the pens when they were still with their dams prior to weaning (*M. sativa*). The *C. argyrea* and *C. podocarpa* were nonexistent at Neudamm camps and were brought in from a different agro-ecological zone, hence goats were not familiar with them and a longer adjustment period was necessary. Diet



selection depends on the sensory stimuli of sight, smell, taste and touch, which are influenced by the previous experience the animal has had with the feed (Holmes et al., 2007). Indeed Distel et al. (1994) have shown that dietary experience early in life can have significant effect on consumption of low quality roughage and diet selection in free-choice situations. Similarly, lambs with previous experience grazing service berry showed greater use of this plant than inexperienced lambs (Flores et al. 1989).

The comparisons of pairs of legumes at individual days (days 5 to 10) in Table 4.3, clearly show a preference of *M. sativa* over *C. argyraea* and *C. podocarpa*. Although there was a steady increase in the amounts of *C. argyraea* and *C. podocarpa* consumed after the adjustment, goats may have preferred *M. sativa* because of its higher nutritive value and absence of post-ingestive adverse effects at the level it was included in the diet. In grazing trials of dairy cows on grasses, legumes and herbs, Horadagoda et al. (2009) reported that the concentration of water soluble carbohydrates (WSC) was strongly related to cow preference for forage species. Other studies, however, showed that other plant aspects for example existence of bitter compounds (e.g. alkaloids in fescue, sesquiterpenes in chicory) (Jones and Roberts, 1991) and high fibre and tensile strength may also influence palatability. *Crotalaria* species contain pyrrolizidine alkaloids (Lucena et al., 2010) and these may have caused aversions, particularly in the adaptation period because of the bitterness.

Reid et al (1992) found significant differences in particle passage rates for some cultivars of switch grass (*Panicum virgatum* L.) which may have influenced intake and hence the differences in consumption evident among the *M. sativa* and the *Clotalaria* species may also be due to differences in particle size, which influences rumen fill. Numerically there appears to be a diminution of differences in amounts consumed between *M. sativa* and the *Clotalaria* species

over time, which possibly indicates the animals evolving a coping mechanism in dealing with the possibly undesirable post-ingestive adverse effects. Similar to the way plants have evolved defensive mechanisms for their protection, many animals, microorganisms and parasites have evolved parallel mechanisms to overcome these defenses (Hartmann and Witte, 1995). Plant secondary metabolites (PSM) or phytochemicals that are ingested interact with tissues, enzymes and other compounds within the animal and this is dependent on the physico-chemical attributes of the compounds involved and their susceptibility to transformation (Acamovic and Brooker, 2005). Ingested PSM can pass through the animal without alteration or combine with bile salts and excreted in the faeces. The PSM can also be either absorbed directly or transformed within the GIT and then absorbed and deposited (Acamovic and Brooker, 2005).

Intriguingly no differences were evident in preferences for *C. argyrea* and *C. podocarpa*, despite indigenous knowledge that indicated Boer goats in the Tsumis area where the *Crotalaria* species are abundant and were harvested had preference for *C. argyrea*. The NDF although not different ( $P > 0.05$ ) was numerically greater in *C. podocarpa*, which partly corroborates the indigenous knowledge on the preference ranking of the two species. It is plausible that chemical composition is altered in hays for the two *Crotalaria* species as opposed to fresh vegetative materials in the veld or the test period may not have been sufficiently long to allow the naive weaned goats without prior exposure, to discern differences in post-ingestive effects for the two *Crotalaria* species.

The objective in palatability or preference studies are usually to establish if rankings of feed are related to chemical composition and their possible utility in predicting potential animal performance (Reid et al., 1992). Preference of sheep for some cultivars of switchgrass was associated with lower NDF and higher N concentration (Reid et al., 1992). On the contrary,

Horadagoda et al. (2009) found a significant negative correlation between nitrate-N concentration and preference; this can induce a spurious negative correlation between CP and preference because CP reflects the concentration of nitrate-N. On the contrary, Ngwa et al (2003) argue that numerous associations reported between plant characteristics and plant palatability to ruminant animals are situation-specific rendering them useless as general selection criteria. These include concentrations of sugars or soluble carbohydrates, protein or nitrogen, fibre or cell wall constituents, ether extracts, individual minerals or total ash, carotene, vitamins, organic acids, tannins and silica (Marten, 1969). Having found that legume, day and legume x day interactions influenced ( $P < 0.05$ ) proportion consumed, we fitted the individual chemical parameters (NDF, ADF, CP and EE) for the days 5 to 10. There was a positive regression of proportion consumed on ADF ( $0.1967 \pm 0.0631$ ,  $P = 0.0035$ ) and a negative regression on ether extract (EE) ( $-0.2721 \pm 0.0786$ ,  $P = 0.0014$ ). In ruminants a high proportion of unsaturated fatty acids will depress fibre digestion in the rumen (McDonald et al., 2010) and may have contributed to the aversion, given that both *Crotalaria* species had high concentrations of EE.

The relationship between preference or palatability and intake is unclear (Gherardi and Black, 1991). Feed preference may not be indicative of potential intake or voluntary intake for different forages (Gherardi et al., 1991; Minson and Bray, 1986). It is believed that palatability is not an important factor influencing intake, except where there are deterrents to consumption (e.g. thorns) or where the food is contaminated for example with excreta (McDonald et al., 2010). Burns et al. (1988) in a trial involving yearling steers grazing a range of *Panicum* accessions found consistent differences in palatability among accessions, but this was unrelated to IVDMD, fiber concentration or agronomic characteristics.

Palatability is influenced by many factors including smells and odours of the forage legume, tannins and other constituents of browse (McDonald et al., 2010). Palatability can be improved by addition of additives for example oil or molasses to multivitamin blocks (McDonald, 2010). Preference tests are useful in the short term because they may influence the quality of the diet consumed and in the long term because of feedback effects on pasture composition under grazing conditions (Hodgson and Illius, 1996). This is all the more important when legumes are introduced in grass swards as to improve herbage yield and animal performance, as it may influence legume persistence. The results in this study, indicate that all the five legumes tested (*O. burchellii*, *C. argyraea*, *C. podocarpa*, *V. lobatifolia* and *M. sativa*) were highly palatable, although an adjustment period is necessary for species to which animals may not have been previously exposed.

Notwithstanding the high palatability observed in the *Crotalaria* species, it should be noted that many of them have bitter leaves due to the presence of toxins such as pyrrolizidine alkaloids, diterpenes and phenolic compounds (Abukutsa-Onyango, 2004). These toxins have been reported to cause toxicity in livestock, although shoots of *C. brevidens* are used as a fodder in livestock and its leaves are consumed as a vegetable. *Crotalaria* sp cause chronic or acute toxic effects in livestock due to pyrrolizidine alkaloids (Lucena et al., 2010). *Crotalaria* sp accumulate monocrotaline in leaves (Rocha et al., 2009) which together with related pyrrolizidine alkaloids are responsible for toxicity in livestock which have eaten them. It is not clear why the goats consumed *C. podocarpa* which contains pyrrolizidine alkaloids and flavonoid glycosides in the aerial parts (Wanjala and Majinda, 1999) and has been reported to be unpalatable and can even cause livestock poisoning (Botha and Penrith, 2008). It is plausible that no overt adverse effects on animal health were detected due to the limited quantities of

*Crotalaria* given, or the exposure duration may have been short (van Wyk et al., 2014) or goats may have adaptive mechanisms that enable them to neutralize the poisonous principles. Research is needed to determine if any antinutrients are found in *Crotalaria agyraea* and its influence on livestock performance.

The relative palatability of any feed depends on the nature of the associated feeds on offer (Marten, 1969), hence it is possible that the effects of any toxins may have been diluted by the ingestion of other feeds. Hill et al. (2009) have also argued that complementary feed selection (mixing) could be beneficial in improving the overall nutritional quality of the diet, enabling the animals to regulate the balance of ingested nutrients.

For animals not accustomed to forages being tested, a palatability test is a useful indicator of relative acceptability (Stewart and Dunsdon, 1998). Smaller quantities of forage material are required compared to a conventional feeding trial (Stewart and Dunsdon, 1998) and use of a standard feed, in this case lucerne serves as a rapid screening test. Compared to other criteria of performance, palatability or preference is difficult to measure mainly because it involves an interaction between plants and animals, both subject to many influences (e.g. season effect for plants and physiological status for animals). Although hand feeding experiments may provide a relative ranking of forages, they have high labour and expense requirements and may involve ‘artificial’ conditions as related to feeding behavior, specifically obviating the need for prehension and selection around (dung and urine) contaminated areas or variables related to canopy (Nombekela et al., 1994). It is also possible that harvesting may have removed olfactory cues of the plant (Horadagoda et al., 2009).

Apparent palatability of each feed is largely dependent on the species composition of the experiment; species that have low palatability should not be rejected, because they may be

consumed if fed alone after some adaptation (Stewart and Dunsdon, 1998). Palatability tests also do not provide information on the extent and ease with which animals adapt to different feeds, which requires study through long-term feeding experiments (Stewart and Dunsdon, 1998). Notwithstanding these limitations, this study has provided some baseline information which forms a basis for managing mixed forage swards to minimize over exploitation of preferred species. All the native legumes tested were palatable indicating long-term feeding experiments can be conducted without concern as to the acceptability of these legumes. Availability of sufficient quantities of materials restricted palatability trials that could be conducted with other legumes (e.g. *P. biflorum*, *L. purpureus* subsp. *uncinatus*, *R. fleckii*, *R. totta* (Thunb.) DC. var. *totta*) whose chemical analyses indicated they are of high nutritive value. Future research should investigate the palatability of these legumes in ruminant diets.

#### 4.5 Conclusions

The relative palatability of the legumes *V. lobatifolia* and *O. burchellii* was similar to *M. sativa*; the *Crotalaria* species required an adaptation period of 3 to 4 days, and their consumption approached but was still less than for the three other forage legumes. Other studies (Reid et al., 1992) have shown that a considerable adaptation period (several weeks) may be required for animals to adapt to high fiber diets. Besides fiber content, there may be antinutritional factors that may necessitate longer adaptation periods in animals not accustomed to feeding certain diets. Although all the four legumes were highly palatable and have high nutritive value, research is needed to identify antinutrients in these legumes before they can be used in ruminant diets. Preference tests for different legume species can be useful in managing mixed species pastures to ensure the dominance of the most desirable species were retained within a given ecological setting.

## CHAPTER 5

### 5 Feeding trial of weaned Boer goats under varying levels of legume supplementation

#### [Objectives (iii) and (iv)]

#### 5.1 Introduction

Feed intake and diet digestibility of poor quality basal grass diets by ruminants can be enhanced by supplementing with legume forages (Minson and Milford, 1967; Getachew et al., 1994). Nitrogen retention by ruminants will be increased when grass diets that do not meet ruminant energy and N requirements are fed legume supplements (Mosi and Butterworth, 1985; Matizha et al., 1997). Exotic legumes were tried in northern Namibia under the Northern Regions Livestock Development Project (NOLIDEP) during the 1996-1997 rainy season, with the legume species *Centrosema pascuorum* and *Aeschynomene Americana* but they had poor performance because they were planted late, the sites were quickly and deeply flooded and the species were poorly adapted (Mukulu and Sweet, 1997).

Lucerne (*Medicago sativa*) is the most widely used forage legume by commercial farmers in Namibia, but is expensive as it is predominantly grown under irrigation. Lablab (*Lablab purpureus*) is under investigation in Namibia for use primarily as a green manure crop (Shaningavamwe K. L., pers. Comm., 2016) although it has a high nutritive value for feeding ruminants and is grown as an annual or short-lived fodder crop on a large scale in Australia, Zimbabwe and Kenya (Adebisi and Bosch, 2004). A number of indigenous legumes exist in rangelands in Namibia, but their potential in contributing to ruminant nutrition has not been exploited. Moreover, changes in land use, selective grazing, rangeland degradation and bush encroachment may threaten survival of some legume species (Muir et al., 2011) and it is therefore important to tap into the wide biodiversity among native legumes, which may provide opportunities for resilience under climate change.

Chemical analyses of a range of indigenous legumes in central Namibia which was part of this study (Experiment 1), indicated they are high in crude protein. Also five (5) of the legumes (*Crotalaria argyrea*, *Crotalaria podocarpa*, *Medicago sativa* [lucerne], *Vigna lobatifolia* and *Otoptera burchellii*) were used in experiment 2, which was a 10-day palatability trial with Boer goats and all five legumes were found to be palatable and could be used to supplement grass hay for feeding goats. Effects of supplementing grass hay with indigenous legume hays on performance of Boer goats are unknown. The objectives of this study were therefore to determine: (i) the voluntary feed intake at different (0%, 18% and 31%) legume supplementation levels (DM basis); (ii) the influence of supplementation with *M. sativa* and *L. purpureus* compared to the native legumes *O. burchellii* and *V. lobatifolia* on the voluntary intake and body weights in Boer goats; and (iii) the ADG and body weights in Boer goats under different (0%, 18% and 31%) legume supplementation levels (DM basis).

## **5.2 Materials and Methods**

### **5.2.1 Feeding of animals and chemical analyses of forages**

The growth trial involved 32 weaned Boer goats (19 females and 13 males), of average age  $11.6 \pm 0.5$  months that weighed  $18.4 \pm 0.7$  kg and that had been vaccinated against internal and external parasites using IVOMEK ® and Ecomintic50 (Merial, South Africa and Afrivet, South Africa, respectively). Information about date of birth, sire, dam, sex, birth type (single or twin), birth weight and weaning weight was also obtained. This experiment involved four (4) forage legume species namely *Vigna lobatifolia*, *Otoptera burchellii*, *Lablab purpureus* (lablab) and *Medicago sativa* (lucerne), which were given as supplements; and a control (no supplement). *V. lobatifolia* and *O. burchellii* were selected on the basis of high palatability as indicated in the palatability experiment and also relative abundance at Neudamm campus farm and along road



sides. *L. purpureus* was selected on the basis of the high biomass yield obtained from a companion study (Kaholongo, 2016). *M. sativa* was selected as a positive control because it is highly nutritious and is commonly used in Namibia.

The legumes *V. lobatifolia* and *O. burchellii* were naturally growing and were harvested from camps at the Neudamm campus of the University of Namibia and along roadsides in the Khomas region. The harvested legumes were dried under shade to minimize nutrient loss and stored under shade. *Otoptera burchellii* forms woody stems as it matures, hence once dried, it was trampled by foot to remove hard stems, leaving leaves and young dried twigs as feeding material. *Lablab purpureus* was grown at Neudamm campus as part of the companion study (Kaholongo, 2016) and was harvested at mid bloom stage and dried under shade. *Medicago sativa* was purchased from commercial suppliers in Windhoek.

During the adaptation period feed was initially offered at about 4% of body weight (Turner et al., 2011) and this was increased by 10% based on the previous day's intake. For simplicity, the legume hays were offered in fixed amounts of 200 and 400 g/goat.day. This effectively led to the following 9 dietary treatment groups (based on % DM legume inclusion) to which goats were randomly assigned by sex: control [C] (0% legume), 18% lablab [LP18], 18% *O. burchellii* [OB18], 18% *V. lobatifolia* [VL18], 18% lucerne [LC18]; 31% lablab [LP31], 31% *O. burchellii* [OB31], 31% *V. lobatifolia* [VL31] and 31% lucerne [LC31]. Each treatment combination was replicated 3 times, except the control which was replicated six (6) times, and OB18 and LP31 which were replicated four (4) times (Table 5.1). In experiments involving comparisons of treatments with controls, it is recommended to use more observations for the control treatment (e.g. na) than for the other treatments (e.g. n); the ratio na/n should be chosen to be approximately equal to the total number (a) of treatments (i.e.  $na/n = a^{0.5}$ ) (Montgomery,

1991). Samples of legume and grass hays were analysed for Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fibre (CF), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Ash.

Goats were housed in individual pens for a total period 74 days of which 14 days was an adaptation period to allow the animals to adjust to both feed and pens. Voluntary feed intake of each animal was determined during the adaptation period. Animals were fed the legume and checked to ensure it was finished before providing the grass hay. Chopped grass hay was provided in a bin *ad libitum* and the amounts given and refusals recorded. During the experimental period (60 days), animals were weighed weekly in the morning prior to feeding, to determine the average daily gain (ADG) and body weights. Water and mineral licks were available *ad libitum*.

The amount of refusals by each animal from the previous day's feeding was collected and weighed daily and pooled into one sample per each 4 days of the experiment (i.e. 0-4, 5-9, 10-14 days, etc.) to determine the average daily feed intake. The pooled refusal was mixed thoroughly and air-dried, and a representative sample was taken and subsequently oven dried for dry matter (DM) determination and ash. Feed intake was expressed as grams DM/day and g DM/kg metabolic weight/day (i.e. g DM/kg  $W^{0.75}$  per day). Feed intake and body weights were treated as weekly repeated measures for the 11 weeks of the trial.

Furthermore a digestibility experiment was carried out with one animal from each treatment group above randomly selected (= 9 goats) and moved from the pens to individual metabolic crates (0.5 x 1.5 m) for 7 days at the beginning of experiment and at 3 weeks intervals thereafter i.e. during weeks 1, 4, 7 and 10. Feed offered during each experimental period was weighed and sampled daily. Feed refusals and feces for each period were collected and stored at

-20°C. Due to unforeseen circumstances the materials from the digestibility experiment, could not be analysed to determine digestibility, but feed intake and body weights of the goats were used in statistical analyses.

**Table 5.1 : The lay out for the feeding and digestibility trial<sup>1</sup>**

Treatment	C	LP18	OB18	VL18	LC18	LP31	OB31	VL31	LC31
Number	(4♀,	(1♀,	(1♀,	(2♀,	(2♀,	(3♀,	(2♀,	(2♀,	(2♀,
of goats	2♂)	2♂)	3♂)	1♂)	1♂)	1♂)	1♂)	1♂)	1♂)

<sup>1</sup>C = Control (0%); LP18 = *L. purpureus* (18%); OB18 = *O. burchellii* (18%); VL18 = *V. lobatifolia* (18%); LC18 = *M. sativa* (18%); LP31 = *L. purpureus* (31%); OB31 = *O. burchellii* (31%); VL31 = *V. lobatifolia* (31%); LC31 = *M. sativa* (31%). ♀ = Female; ♂ = Male

### 5.2.2 Statistical analyses

The data were checked for normality and outliers before analysis. One goat had much reduced feed intake and had lost about 5 kg by the end of the trial period and was excluded from the analysis. Data were analysed by Proc Mixed (SAS, 2008). The model for voluntary feed intake on dry matter basis (VFI), voluntary feed intake on metabolic body weight basis (VFIMBW), voluntary feed intake of basal grass hay on metabolic weight basis (BASALMBW) and voluntary feed intake of legume hay on metabolic body weight basis (LEGUMEMBW) included sex, birth type (single or twin), treatment, week, treatment x week and treatment x sex effects. The appropriate covariance structure was selected based on BIC criterion (Littell et al., 1998). Average daily gain (ADG) was analysed by the GLM procedure (SAS, 2008) with treatment and initial weight fitted in the model. Post-Hoc analysis could not separate treatment effects on ADG, therefore weights at each week during the trial (weeks 3 – 11) were modeled as repeated measures using Proc Mixed (SAS, 2008) which takes into account correlation due to a

common contribution from the animal and also time trends, whereby measurements close in time may be more correlated than those far apart (Littell et al., 1998). Means were separated by a PDIFF statement. Significance was declared at  $P < 0.05$ ; trends were accepted if  $0.05 < P < 0.10$ . With the exception of ADG, the analyses were done on all available data (weeks 1 – 11).

## 5.3 Results

### 5.3.1 Effect of legume supplementation on voluntary feed intake (VFI)

The univariate statistics for the different variables studied are shown in Table 5.2. Average daily gain showed the highest variability, ranging from  $-43.9$  to  $54.4$  g/day and with a coefficient of variation of 153%. The mean daily intakes and refusals are shown in Table 5.3. Realized mean forage legume dietary intakes (%) for the low (18% legume inclusion) group was 22.5% and 36.8% for the high (31% legume inclusion) group. Refusals varied from 15.9% to 21.4% DM.

**Table 5.2 :** Univariate statistics for the variables studied in the feeding trial with weaned Boer goats (weeks 1 - 11)<sup>1</sup>

Variable	n	Minimum	Maximum	Mean	s.d*	CV
Initial Age (months) <sup>2</sup>	32	5.3	17.3	11.1	2.8	25.5
Adjustment period weight (kg) <sup>3</sup>	32	11.6	28.8	19.3	4.0	20.5
Starting weight (kg) <sup>4</sup>	32	11.2	26.4	18.4	3.8	20.4
End weight (kg)	32	11.6	26.0	19.4	3.2	16.4
ADG (g/day)	31	-37.8	28.4	3.8	17.8	468.8
VFI (g DM/day) <sup>5</sup>	352	399.6	1203.3	851.4	143.1	16.8
Basal_VFI (g DM/day) <sup>6</sup>	352	317.8	833.7	630.6	77.8	12.3
Legume_VFI (g DM/day) <sup>7</sup>	352	0.0	374.4	220.8	134.4	60.9
VFIMBW (g DM/kg W <sup>0.75</sup> /day) <sup>8</sup>	352	42.7	166.3	95.8	21.5	22.5
BASALMBW (g DM/kg W <sup>0.75</sup> /day) <sup>9</sup>	352	42.1	107.2	70.7	12.6	17.8
LEGUMEMBW (g DM/kg W <sup>0.75</sup> /day) <sup>10</sup>	352	0	60.4	25.1	15.8	63.1

<sup>1</sup>Note: we denote the weeks as follows: weeks 1 – 2 = adjustment period (14 days); weeks 3 – 11 = trial period (60 days);

<sup>2</sup>Initial age = age of goats (months) at beginning of trial (week 1).

<sup>3</sup> Adjustment period weight (kg) = Weight of the goats at week 1.

<sup>4</sup>Starting weight = weight of goats at week 3.

<sup>5</sup> VFI = Voluntary feed intake;

<sup>6</sup>Basal\_VFI = Voluntary feed intake for basal grass hay;

<sup>7</sup>Legume\_VFI = Voluntary feed intake for legume supplement; <sup>8</sup>VFIMBW = Voluntary feed intake per unit of metabolic body weight per day;

<sup>9</sup>BASALMBW = Voluntary feed intake of basal grass hay per unit of metabolic body weight per day; <sup>10</sup>LEGUMEMBW = Voluntary feed intake of legume supplement per unit of metabolic body weight per day. \* s.d = standard deviation.

**Table 5.3:** Mean (s.d.) daily forage hay intake and refusals of weaned Boer goats<sup>1</sup>

Treatment	C	LP18	OB18	VL18	LC18	LP31	OB31	VL31	LC31
DLI (g DM/goat)	-	179.2 ± 13.7	184.7 ± 4.3	181.7 ± 11.2	183.3 ± 8.8	362.4 ± 18.1	368.3 ± 9.8	369.2 ± 6.6	357.4 ± 39.7
DGI (g DM/goat)	666.3 ± 70.2	645.2 ± 77.7	618.0 ± 85.9	630.0 ± 77.5	618.9 ± 73.5	623.3 ± 75.3	622.2 ± 63.4	644.0 ± 71.0	609.9 ± 77.3
DLO (g DM/goat) <sup>2</sup>	-	185.4	186.8	186.2	187.2	370.8	373.6	372.4	374.4
DGO (g DM/goat)	839.6 ± 98.4	832.9 ± 105.3	836.2 ± 102.0	832.9 ± 105.3	833.2 ± 104.1	836.2 ± 102.0	832.9 ± 105.3	832.9 ± 105.3	832.9 ± 105.3
Refusals (%DM) <sup>3</sup>	20.6	19.0	21.4	20.3	21.4	18.3	17.9	15.9	19.9
Legume refusal (%DM) <sup>4</sup>	0	3.2	0.9	2.1	1.8	3.8	2.4	1.6	7.1

<sup>1</sup>s.d. = standard deviation; DLI = Daily legume intake; DGI = Daily grass intake; DLO<sup>2</sup> = Daily legume offered; DGO = Daily grass offered; <sup>2</sup> The amount of legume hay offered was kept fixed at 0, 200, 400 g per goat per day for control, 18% and 31% inclusion levels, respectively.<sup>3</sup>Expressed as a percent of the diet offered on a DM basis. <sup>4</sup> Legume contribution to refusal (% DM).

Voluntary feed intake (VFI) on dry matter basis was affected ( $P < 0.0001$ ) by week and treatment. VFI was greater ( $P < 0.0001$ ) in goats fed legume hay supplements than controls. At the 18% inclusion level, VFI of LP18 exceeded ( $P < 0.0037$ ) that of OB18 and VL18; VFI of

LP18 did not differ ( $P > 0.05$ ) from that of LC18 (Table 5.4); VFI of LC18 tended ( $P = 0.0584$ ) to be higher than that of VL18. Hence in general the ranking of legumes on VFI at 18% inclusion level is: {LP18, LC18} > {OB18, VL18}.

The VFI of goats fed 31% legume hay supplement exceeded ( $P < 0.0001$ ) that of goats supplemented at 18% level. At the 31% supplementation level, VFI under VL31 exceeded ( $P = 0.0056$ ) that under LC31; VFI under OB31 tended ( $P = 0.0920$ ) to be higher than LC31. The VFI under LP31 did not differ ( $P > 0.05$ ) from that under OB31 or VL31; the VFI under LP31 tended ( $P = 0.0556$ ) to be higher than that under LC31. Hence in general the ranking of legumes on VFI at 31% inclusion level is: {VL31, OB31, LP31} > LC31. Pre-planned contrasts of the VFI show that at the 18% inclusion level, *L. purpureus* and *M. sativa* are superior to the indigenous species (*O. burchellii* and *V. lobatifolia*); at the 31% inclusion level, there is no difference ( $P > 0.05$ ) of between *L. purpureus* and *O. burchellii* or *L. purpureus* and *V. lobatifolia*; *M. sativa* generally tends to be inferior to *L. purpureus* and the indigenous legumes (Table 5.6).

The least squares means for VFI varied by week (Table 5.5). The VFI increased reaching a peak at week 4, then fluctuated thereafter. There was evidence for variance heterogeneity across weeks, for instance the variance for weeks 8 and 5 were 12802 and 11053, respectively, which were at least 11 times the smallest variance 1006.84 (week 1).

**Table 5.4:** Least squares means ( $\pm$  S.E) of voluntary feed intake (VFI) (g DM) of weaned Boer goats as influenced by treatment <sup>1</sup>

<b>Treatment</b>	<b>g DM/day</b>	<b>S.E</b>
Control (0%)	668.7 <sup>a</sup>	8.8
<i>L. purpureus</i> (18%)	821.2 <sup>c</sup>	10.8
<i>O. burchellii</i> (18%)	783.1 <sup>b</sup>	9.9
<i>V. lobatifolia</i> (18%)	777.7 <sup>bd</sup>	10.8
<i>M. sativa</i> (18%)	802.8 <sup>cd</sup>	10.8
<i>L. purpureus</i> (31%)	994.4 <sup>f</sup>	9.9
<i>O. burchellii</i> (31%)	992.8 <sup>f</sup>	10.8
<i>V. lobatifolia</i> (31%)	1009.2 <sup>f</sup>	10.8
<i>M. sativa</i> (31%)	970.6 <sup>e</sup>	10.8

<sup>1</sup>S.E – Standard error; Legume supplementation levels are in brackets.

Least squares means within a column with different superscripts differ ( $P < 0.05$ )

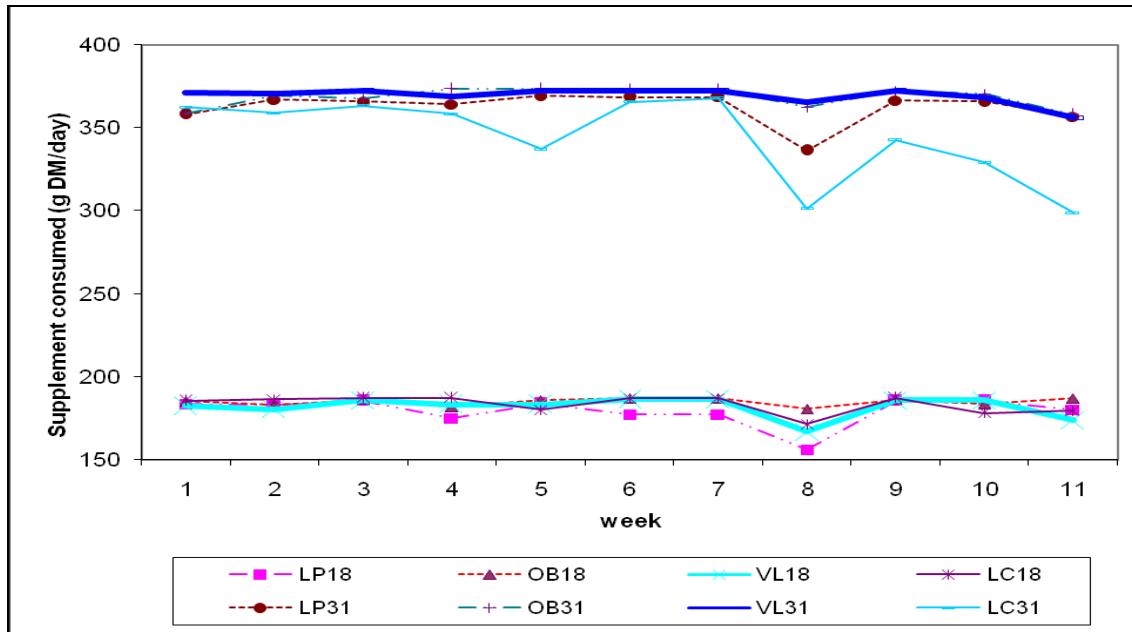


**Table 5.5:** Least squares means ( $\pm$  S.E) of voluntary feed intake (VFI) (g DM) of weaned Boer goats as influenced by week

Week	g DM/day	S.E
1	906.4 <sup>e</sup>	5.6
2	873.5 <sup>bd</sup>	8.9
3	888.2 <sup>cd</sup>	6.2
4	964.6 <sup>f</sup>	12.9
5	865.6 <sup>bd</sup>	18.6
6	884.0 <sup>cd</sup>	11.5
7	909.8 <sup>e</sup>	12.8
8	798.2 <sup>a</sup>	20.0
9	796.0 <sup>a</sup>	6.5
10	823.8 <sup>a</sup>	7.6
11	848.3 <sup>b</sup>	9.8

Least squares means within a column with different superscripts differ ( $P < 0.05$ )

Consumption of the legume hay supplement was influenced ( $P < 0.05$ ) by treatment, week and treatment x week interactions. The interaction was mainly due to a drop in consumption of legume supplement which occurred at weeks 5 and 8, but this was more pronounced in LC31 (Figure 5.1). Following the drop in week 8, there was continued wide fluctuation in LC31 consumed.



**Figure 5-1 :** Least squares means of treatment by week interactions for supplement consumed (g DM/day)

Consumption of basal grass hay on DM basis was influenced ( $P < 0.05$ ) by treatment, week and type of birth (singles or twins). Supplementation at 31% resulted in greater ( $P = 0.0011$ ) consumption of grass hay than at 18%. The least squares means ( $\pm$  s.e.) (g DM) for basal grass hay consumed for the treatments C, LP18, OB18, VL18, LC18, LP31, OB31, VL31 and LC31 were:  $663.6 \pm 7.4$ ,  $646.1 \pm 9.3$ ,  $606.2 \pm 8.3$ ,  $607.0 \pm 9.0$ ,  $606.3 \pm 9.0$ ,  $634.0 \pm 8.2$ ,  $633.2 \pm 9.0$ ,  $647.0 \pm 9.0$  and  $624.5 \pm 9.3$ , respectively. The grass hay consumption was greatest in the control diet (C) and this showed a trend to be higher than LP18 ( $P = 0.0604$ ) and also VL18 ( $P = 0.0684$ ).

Consumption of grass hay increased up to week 4 and then fluctuated thereafter, similar to the trends observed for VFI. The least squares means ( $\pm$  s.e.) (g DM) for grass hay consumed for the weeks 1 – 11 were:  $666.1 \pm 5.2$ ,  $631.9 \pm 8.7$ ,  $645.0 \pm 6.2$ ,  $723.7 \pm 11.8$ ,  $625.1 \pm 17.6$ ,  $640.5 \pm 11.1$ ,  $666.1 \pm 12.5$ ,  $572.4 \pm 17.1$ ,  $554.3 \pm 6.5$ ,  $585.3 \pm 5.8$  and  $617.1 \pm 6.8$ , respectively.

Examination of the variances in basal feed consumed showed that the greatest variance was in week 5 (9922.15) and week 8 (9332.73) and least variation was in week 1 (816.95). Goats born as singles had higher intake of grass hay than twins ( $635.4 \pm 6.8$  vs.  $624.2 \pm 6.1$ ).

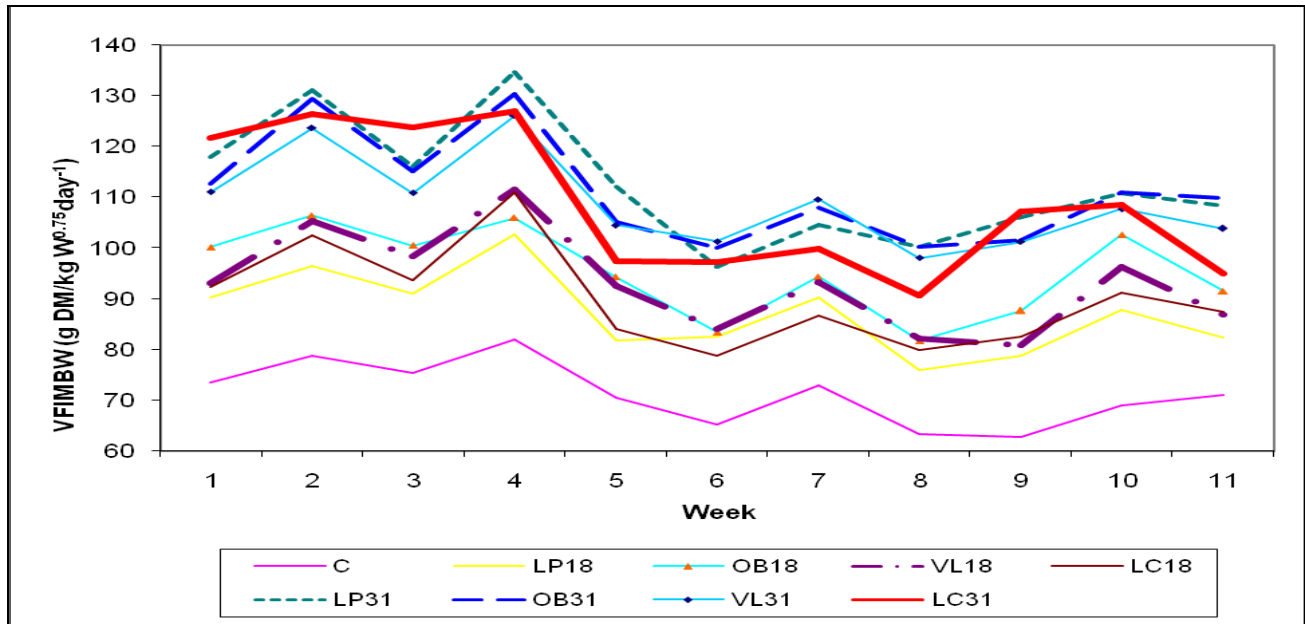
**Table 5.6:** Estimates of differences between least squares means ( $\pm$  S.E) of VFI (g DM) under different supplementation regimes for weaned Boer goats, averaged over 11 weeks of the trial.

Comparison	Estimate	S.E	P-value
Control – <i>L. purpureus</i> (18%)	-152.5	10.9	< 0.0001
Control – <i>O. burchellii</i> (18%)	-114.4	10.0	< 0.0001
Control – <i>V. lobatifolia</i> (18%)	-109.0	10.9	< 0.0001
Control – <i>M. sativa</i> (18%)	-134.1	10.9	< 0.0001
<i>L. purpureus</i> (18%) – <i>O. burchellii</i> (18%)	38.1	11.8	0.0037
<i>L. purpureus</i> (18%) – <i>V. lobatifolia</i> (18%)	43.6	12.6	0.0021
<i>L. purpureus</i> (18%) – <i>M. sativa</i> (18%)	18.5	12.6	0.1560
<i>O. burchellii</i> (18%) – <i>V. lobatifolia</i> (18%)	5.4	11.8	0.6490
<i>O. burchellii</i> (18%) – <i>M. sativa</i> (18%)	-19.7	11.8	0.1089
<i>V. lobatifolia</i> (18%) – <i>M. sativa</i> (18%)	-25.1	12.6	0.0584
<i>L. purpureus</i> (31%) – <i>O. burchellii</i> (31%)	1.6	11.8	0.8924
<i>L. purpureus</i> (31%) – <i>V. lobatifolia</i> (31%)	-14.8	11.8	0.2223
<i>L. purpureus</i> (31%) – <i>M. sativa</i> (31%)	23.8	11.8	0.0556
<i>O. burchellii</i> (31%) – <i>V. lobatifolia</i> (31%)	-16.4	12.6	0.2060
<i>O. burchellii</i> (31%) – <i>M. sativa</i> (31%)	22.1	12.6	0.0920
<i>V. lobatifolia</i> (31%) – <i>M. sativa</i> (31%)	38.5	12.6	0.0056
<i>L. purpureus</i> (18%) – <i>L. purpureus</i> (31%)	-173.2	11.8	< 0.0001
<i>V. lobatifolia</i> (18%) – <i>V. lobatifolia</i> (31%)	-231.5	12.6	< 0.0001
<i>M. sativa</i> (18%) – <i>M. sativa</i> (31%)	-167.9	12.6	< 0.0001
<i>O. burchellii</i> (18%) – <i>O. burchellii</i> (31%)	-209.7	11.8	< 0.0001

### 5.3.2 Effect of legume supplementation on Voluntary feed intake on metabolic body weight basis (VFIMBW)

The voluntary feed intake on metabolic body weight basis (VFIMBW) ( $\text{g DM/kg W}^{0.75}\text{day}^{-1}$ ) was influenced ( $P < 0.05$ ) by treatment and week; there was a trend for sex ( $P = 0.0777$ ) and treat x week interactions ( $P = 0.0704$ ) to also influence it. Averaged over 11 weeks, VFIMBW was greater ( $P < .0006$ ) at the higher inclusion level (31%) than at the lower inclusion level (18%). Averaging over all 11 weeks, supplementation increased ( $P < 0.05$ ) VFIMBW over non-supplementation for *O. burchellii*, *V. lobatifolia* and Lucerne; only a trend ( $P = 0.070$ ) for increased VFIMBW of LP18 over control (C) was observed. The VFIMBW tended to be higher for females than males ( $99.0 \pm 2.2$  vs.  $96.10$ ;  $P = 0.0777$ ).

Due to existence of significant interactions, treatment effects needed to be compared for each week separately. There was a declining trend in VFIMBW over time (Figure 5.2). There were no differences ( $P > 0.05$ ) in VFIMBW among legume species at 18% or 31% legume inclusion level. Pre-planned contrasts, however, show differences in VFIMBW for comparison within a given legume. Supplementation at 31% led to higher VFIMBW compared to 18% as follows: (LP35 vs. LP18 at weeks 1 – 4;  $P < 0.05$ ); (LC31 vs. LC18 at weeks 1 – 4;  $P < 0.05$ ); (OB31 vs. OB18 at week 4;  $P = 0.0093$ ); (VL31 vs. VL18 at week 4;  $P = 0.0085$ ). Trends for higher VFIMBW at 31% compared to 18% legume inclusion were also observed for the following: (VL31 vs. VL18 at week 2;  $P = 0.0592$ ); (LP31 vs. LP18 at weeks 5, 10 and 11;  $P < 0.10$ ); (OB31 vs. OB18 at week 2;  $P = 0.0599$ ).



**Figure 5-2:** Least square means of treatment by week interactions for VFIMBW (g DM/kg W<sup>0.75</sup>day<sup>-1</sup>)

Week influenced ( $P < 0.0001$ ) intake of grass hay on metabolic body weight basis (BASALMBW); treatment showed a trend ( $P = 0.0784$ ). Averaged over the 11 weeks, BASALMBW for the control exceeded ( $P < 0.05$ ) the supplemented groups. The BASALMBW under 18% supplementation did not differ ( $P > 0.05$ ) from that under 31% legume inclusion level. There was a tendency ( $P = 0.0784$ ) for BASALMBW to increase up to week 4, with fluctuations thereafter.

Legume intake expressed on metabolic body weight basis (LEGUMEMBW) was influenced ( $P = 0.0001$ ) by treatment, week, sex and treatment x week interactions. Female goats had higher ( $P = 0.0001$ ) LEGUMEMBW than males. Trends in LEGUMEMBW (results not shown) for the different treatments over time, mirrored those for VFIMBW (Figure 5.2) and supplement consumed on dry matter basis (Figure 5.1). Treatment LC31 showed the greatest variability in LEGUMEMBW (CV = 11.2% compared to the range 7.0% – 8.9% for the other

treatments). The treatment x week interactions for LEGUMEMBW appear to stem from the wide fluctuations in LC31 from around week 4 onwards.

### 5.3.3 Effect of legume supplementation on average daily gain and weights of Boer goats

Treatment effects did not influence ( $P > 0.05$ ) average daily gain (ADG), but by splitting the treatments into two categories (control and supplemented), a significant treatment effect (hereby called TRT2) was detected. Average daily gain (ADG) was influenced by starting weight ( $P < 0.0021$ ) and TRT2 ( $P = 0.0385$ ) and the R-square for the model was 38.6%. The estimated intercept was  $53.4 \pm 14.1$  ( $P = 0.0007$ ) and the estimated regression coefficient of ADG on starting weight was  $-2.486 \pm 1.073$  ( $P = 0.0021$ ). The least squares means ( $\pm$  S.E.) of ADG for the control group was  $-9.1 \pm 6.5$  and for the supplemented group was  $6.3 \pm 2.8$  (g/day).

Weights of Boer goats were influenced ( $P < 0.05$ ) by birth type, treatment, week and sex x birth type interactions; sex effect showed a trend ( $P = 0.0801$ ) on body weights. Goats born as singles were considerably heavier than twins ( $20.5 \pm 0.7$  vs.  $17.4 \pm 0.7$  kg,  $P < 0.0001$ ). The differences of least squares means for the different sex x birth type interactions are shown in Table 5.7. There were no differences ( $P > 0.05$ ) in weights among females born as singles or twins, but differences ( $P < 0.05$ ) occurred among males based on birth type. Goats tended to gain weight from weeks 2 to 6, followed by decline in weights in weeks 7 to 10 (Table 5.8). The body weight least squares means for the goats (kg) for the control (C), LP18, OB18, VL18, LC18, LP31, OB31, VL31 and LC31 were:  $19.2 \pm 0.8$ ,  $22.1 \pm 0.9$ ,  $17.6 \pm 0.9$ ,  $18.1 \pm 0.9$ ,  $16.4 \pm 0.9$ ,  $18.9 \pm 0.8$ ,  $16.6 \pm 0.9$ ,  $21.1 \pm 0.9$  and  $20.4 \pm 0.9$ , respectively.

Averaging over all 11 weeks, there was no difference ( $P > 0.05$ ) in weights of goats supplemented at 18% or 31% inclusion level. Pre-planned contrasts (Table 5.9), however, show that at the 18% legume inclusion level only *L. purpureus* was clearly superior over the control (estimated difference =  $-2.816 \pm 0.8426$  kg;  $P < 0.0032$ ); Lucerne was inferior ( $P = 0.0019$ ) to the control. *Lablab purpureus* was superior ( $P < 0.05$ ) to *O. burchellii*, *V. Lobatifolia* and *O. burchellii*. Hence at the 18% inclusion level, the ranking was LP18 > VL18 > {OB18, LC18}.

At the 31% inclusion level, pre-planned comparisons show that both *V. lobatifolia* and *M. sativa* were superior ( $P < 0.05$ ) to *L. purpureus* and *O. burchellii*; there was no difference ( $P > 0.05$ ) between *V. lobatifolia* and *M. sativa*. *Lablab purpureus* was superior ( $P = 0.0133$ ) to *O. burchellii*. Hence ranking the legumes at the 31% inclusion level were: {VL31, LC31} > LP31 > OB31. Comparison of the 18% versus the 31% inclusion levels for a given legume species, indicated that *L. purpureus*, *V. lobatifolia* and *M. sativa* were advantageous when included at 18%; on the contrary, *O. burchellii* resulted in better body weights when included at the higher level in the diet (31%).

**Table 5.7:** Differences of least squares means ( $\pm$  S.E) of weights (kg) of weaned Boer goats for different sex x birth type interactions<sup>1</sup>

Sex	Birth type	Sex	Birth type	Estimate	S.E	P-value
Female	Single	Female	Twin	0.1078	0.750	0.8872
Female	Single	Male	Single	-3.8662	0.853	0.0002
Female	Single	Male	Twin	2.1161	0.893	0.0279
Female	Twin	Male	Single	-3.9740	0.530	< 0.0001
Female	Twin	Male	Twin	2.0083	0.663	0.0066
Male	Single	Male	Twin	5.9823	0.817	0.0001

<sup>1</sup> Comparisons should contrast entries in first two columns with next two columns within a row e.g. taking the first row: the estimated difference of females born as singles to females born as twins is  $0.1078 \pm 0.75$  and the difference is not significant ( $P = 0.8872$ ).

**Table 5.8:** Least squares means ( $\pm$  S.E) of weights (kg) of weaned Boer goats as influenced by week

Week	Weight (kg)	S.E
1	19.1 <sup>c</sup>	0.7
2	16.4 <sup>a</sup>	0.7
3	18.2 <sup>c</sup>	0.7
4	17.5 <sup>b</sup>	0.7
5	19.6 <sup>d</sup>	0.7
6	22.0 <sup>f</sup>	0.7
7	20.3 <sup>e</sup>	0.7
8	19.7 <sup>d</sup>	0.5
9	18.7 <sup>c</sup>	0.6
10	17.4 <sup>b</sup>	0.7
11	19.2 <sup>c</sup>	0.6

Least square means within a column with different superscripts differ ( $P < 0.05$ )



**Table 5.9:** Estimates of differences between least squares means ( $\pm$  S.E) for pre-planned contrasts of weights of weaned Boer goats (kg) under different supplementation regimes

<b>Contrast</b>	<b>Estimate</b>	<b>S.E</b>	<b>P-value</b>
Control – <i>O. burchellii</i> (18%)	1.6553	0.7801	0.0032
Control – <i>V. lobatifolia</i> (18%)	1.1481	0.7948	0.1641
Control – <i>L. purpureus</i> (18%)	-2.8160	0.8426	0.0032
Control – <i>M. sativa</i> (18%)	2.8351	0.7948	0.0019
<i>L. purpureus</i> (18%) – <i>O. burchellii</i> (18%)	4.4714	1.0105	0.0003
<i>L. purpureus</i> (18%) – <i>V. lobatifolia</i> (18%)	3.9642	1.0041	0.0008
<i>L. purpureus</i> (18%) – <i>M. sativa</i> (18%)	5.6511	1.0041	<.0001
<i>O. burchellii</i> (18%) – <i>V. lobatifolia</i> (18%)	-0.5072	0.8620	0.5628
<i>O. burchellii</i> (18%) – <i>M. sativa</i> (18%)	1.1797	0.8620	0.1863
<i>V. lobatifolia</i> (18%) – <i>M. sativa</i> (18%)	1.6869	0.8907	0.0728
<i>L. purpureus</i> (31%) – <i>O. burchellii</i> (31%)	2.3194	0.8537	0.0133
<i>L. purpureus</i> (31%) – <i>V. lobatifolia</i> (31%)	-2.1554	0.8737	0.0228
<i>O. burchellii</i> (31%) – <i>V. lobatifolia</i> (31%)	-4.4748	0.9695	0.0002
<i>O. burchellii</i> (31%) – <i>M. sativa</i> (31%)	-3.8163	0.9314	0.0006
<i>V. lobatifolia</i> (31%) – <i>M. sativa</i> (31%)	0.6585	0.9251	0.9251
<i>L. purpureus</i> (31%) – <i>M. sativa</i> (31%)	-1.4970	0.8840	0.1059
<i>L. purpureus</i> (18%) – <i>L. purpureus</i> (31%)	3.1317	0.9521	0.0037
<i>V. lobatifolia</i> (18%) – <i>V. lobatifolia</i> (31%)	4.4250	1.5713	0.0146
<i>M. sativa</i> (18%) – <i>M. sativa</i> (31%)	5.1884	1.7449	0.0108
<i>O. burchellii</i> (18%) – <i>O. burchellii</i> (31%)	-2.7011	1.5181	0.0986

## 5.4 Discussion

The results obtained led to the rejection of the hypothesis of equality in VFI among the 9 different treatments. The response to supplementation, however, depended on the quality of the basal grass hay. The grass hay used in weeks 1 – 4 of the trial consisted predominantly of *Antephora pubences* (75%) and *Microhloa caffra* (25%). In weeks 5 – 9 of the trial, *Stipagrostis uniplumis*, poor quality grass hay was predominantly used because of the drought that was experienced in Namibia in 2015 and we had to rely on what was available in stock from the commercial suppliers of hay. Indeed there was a 10% drop in VFI from week 4 to week 5, which can be attributed to introduction of the poor quality grass hay. The nutritive quality of the grass hay that was used varied and was generally of acceptable quality in weeks 1 – 4 and of poorer quality in weeks 5 – 9. The NDF (%) of the grass hay available in weeks 5 – 9 was much higher than in weeks 1 – 4 ( $58.5 \pm 0.8$  vs.  $52.9 \pm 6.3$ ), reducing the diet digestibility. Sensing the reduction in diet digestibility, the goats compensated for this through reduction in consumption of grass hay, while maintaining constant the legume intake. Feed with high NDF has high fill effects resulting in distention in the reticulorumen, which reduces VFI (Allen, 1996). The proportion of forage legume consumed was higher than what had been assigned by 3.7 – 6.5%, which may be attributed to the poor quality of the grass hay especially in weeks 5 – 9; this might have led to negative effects on ADG (Devincenzi et al., 2014).

Forage intake is affected by a number of factors including fill, rate of digestion and passage rate (Ferrell et al., 1999). Rates of microbial growth and fermentation have a substantial effect on these factors. Limitation of microbial growth is likely in low quality forages, due to low ruminal ammonia concentrations (Ferrell et al., 1999). The CP (% DM) for the grasses used in

the feeding trial varied from  $6.2 \pm 2.0$  to  $6.7 \pm 2.5$ , which is below the 7% minimum threshold for a response to supplementation in a basal diet (Mathis et al., 2000). The results in Table 5.4 are in conformity with the increased VFI when poor-quality forages are supplemented with legume hays and this is attributed to increased supply of Nitrogen which permits increased activity of rumen microbes, reducing rumen-fill and hence enhanced intake (McDonald et al., 2010).

The increased DM intake of poor quality forages as a result of supplementation with legumes is consistent with other studies (Said and Tolera, 1993; Nolte et al., 2003; Foster et al., 2009; Piaggio et al., 2015), especially when the CP content of the forage is less than 60 to 80 g/kg DM (Kartchner, 1980; Matejovsky and Sanson, 1995). The response to RDP supplementation of cattle fed low- to medium quality forages with CP content below the 7% threshold, however, have been variable (Heldt, 1998). The characteristics of the forage being fed (e.g. inherent fermentability and protein availability) and the nutrient status of the animal (e.g. relative protein demand) imposes limits to how responsive intake will be to provision of RDP (Koster et al., 1996). In this study, the rate of passage of digesta from the reticulorumen may also have been higher in the supplemented diets than the control, resulting in higher VFI in the former. The faster degradation and passage rates of legumes contribute to increased feed intake (Mertens, 1973; Reid et al. 1988). It has also been reported that protein supplementation increases digestibility of organic matter and NDF and N intake and flows of Non ammonia N (NAN) without affecting the flow of microbial N (Bargo et al., 2003).

Inclusion of legumes at both 18% and 31% levels increased intake, however, at the higher level, the increase was mainly because of higher intake of the legume and not the grass

hay *per se*. Under natural grazing conditions, animals select a diet that is of higher nutritional value than what is available (Holmes et al., 2007; Hardison et al., 1954); in this study, goats no doubt were able to preferably consume most of the more nutritious legumes while keeping the amount of basal feed consumed fairly constant. It has been reported that in plots consisting of adjacent grass and clover, ruminants had preferences of legume to grass in the ratio 75:25 (Rutter, 2006). It has been reported in studies that used Lucerne hay, that at supplementation levels equal to or above 25% of the total dry matter intake (DMI), substitution effects of Lucerne hay for low quality roughage occur (Hunt et al., 1988; Haddad, 2000; Abate and Melaku, 2009; Zhuoga et al., 2016). In this study by the reduction in basal feed observed in all supplemented groups, it is evident substitution occurred, which can be attributed to the faster degradation and passage rate of legumes (Mertens, 1973; Reid et al. 1988).

Voluntary feed intake of male goats was greater than female goats and this is related to the fasting metabolism per unit metabolic weight being higher in males than females (McDonald et al., 2010). At the 18% inclusion level, the similar VFI under *M. sativa* and *L. purpureus* can be explained by the similarity in NDF ( $36.0 \pm 1.8$  vs.  $39.2 \pm 3.1$ , respectively) and the CP values ( $22.6 \pm 1.2$  vs.  $23.0 \pm 2.1$ , respectively). By contrast, VFI at the 18% inclusion level was greater under *L. purpureus* compared to *O. burchellii* and this may be explained by the differences in NDF ( $39.2 \pm 3.1$  vs.  $44.2 \pm 1.7$ , respectively). A lower NDF implies greater digestibility of the legume, hence reducing rumen fill and enhanced feed intake. Surprisingly, however, at the 18% inclusion level, there was just a trend ( $P = 0.0584$ ) in VFI between Lucerne and *V. lobatifolia* despite *V. lobatifolia* having a higher NDF content ( $44.4 \pm 1.9$ ). This implies NDF alone is insufficient to account for the increased VFI under *V. lobatifolia* supplementation; perhaps there

are differences in physically effective NDF (Galvani et al., 2014) between the legumes, despite having the same NDF.

Rate of removal of particles from the reticulorumen is one of the factors that limits voluntary feed intake. The removal rate is linked to forage chemical composition, particle size, the digestion rate of the digestible content and the clearance of the indigestible fill (McDonald et al., 1991; Allen, 2000). Differences among forage legumes in a number of these characteristics may explain the observed variation in VFI. Passage rate is more important than digestion rate in determining rumen fill (Mertens, 1973) and given the similarity in VFI of *V. lobatifolia* to Lucerne at 18% and even higher intake at 31% legume inclusion despite *V. lobatifolia* having higher NDF, suggests it particles are removed at a faster rate from the reticulorumen, enhancing intake. This is further supported by similar intake of *V. lobatifolia* to *L. purpureus* which has a lower NDF concentration. Hence although alfalfa NDF is known to have a low fill effect (Jung and Allen, 1995), *V. lobatifolia* may even be lower in that respect.

At the 31% inclusion level, OB31 resulted in higher VFI compared to LC31. The seemingly better stimulatory effect of OB31 relative to lucerne, should, however, be viewed cautiously, because unlike the other forage legumes which were fed whole with their leaves and stems, mature stems were separated from the young twigs and leaves in *O. burchellii* and discarded, hence reducing indigestible material with high lignin content. Voluntary feed intake at the 31% legume inclusion level for Lucerne was significantly lower than under supplementation with *L. purpureus*, *O. burchelli* and *V. lobatifolia*. Lucerne contains saponins which are antinutrients (McDonald et al., 2010) and may induce subclinical bloat, thus reducing intake of the grass hay when this legume is included at high levels in the diet. Similar to these results,

Maughan et al. (2014) reported in a trial involving grass-legume pasture mixes, that cattle grazed tall fescue grass more in tall fescue-alfalfa plots than in tall fescue-sainfoin plots, possibly as a means of reducing incidence of bloat.

Pure saponins and saponin-containing plants or extracts have inhibitory effects on protozoa (defaunation) (Patra and Saxena, 2009), but the susceptibility of the protozoa varies, which is an indication that the range of saponins might be dependent on the type of saponins and the protozoal species and may also be diet-dependent. Lu and Jorgensen (1987) observed a diet-dependent response to saponins on bacterial synthesis, whereby the efficiency of bacterial protein synthesis and bacterial protein flow to the duodenum were decreased by lucerne saponins in a roughage-based diet, but had no effect in a concentrate-based diet. It is plausible that at the 31% inclusion level, bacterial activity was reduced, hence contributing to rumen-fill which reduced feed intake. Saponins decrease the passage rate of digesta from the rumen (Lu and Jorgensen, 1987), which may increase the ruminal degradation. The positive effects of saponins on the digestibility of feeds in some studies might be a consequence of the increased bacterial populations, whereas negative effects reported in other studies are due to decreased hydrolytic activities from protozoa and/or bacteria and fungi (Patra and Saxena, 2008).

The significant treatment by week interaction for the amount of legume supplement consumed was due to a drop in consumption of all legumes, but which was more pronounced in treatment LC31. It is not clear to us what might have caused this drop, but the temperatures rose due to transition from the cooler weather (in May to August) to warmer weather in (late September to October), which coincided with the timing of the feeding experiment, hence depressing intake.

Treatment effects were not significant ( $P > 0.05$ ) in influencing ADG. Similarly, Boer x Spanish kids that were on a basal grass hay supplemented with Lablab, *Leucaena leucocephala* and *Desmanthus bicornutus* plus 200 g corn also resulted in just a tendency ( $P < 0.1$ ) for better performance with *Leucaena leucocephala* compared to *Desmanthus bicornutus*, which was attributed to higher CP content (27.5%) in *Leucaena* since voluntary feed intakes were similar among the diets (Kanani et al., 2006). On the other hand, Devincenzi et al. (2014) reported in an experiment involving lambs group-fed on cooksfoot pasture and supplemented with fresh cut Lucerne, ADG of 136, 147, 191 and 224 g/day for diets with increasing levels of Lucerne (0, 25, 50 and 75%, respectively) on DM basis. In this study despite the fluctuations in VFI as a result of changes in quality of basal grass hay offered, the ADG (g/day) as estimated by the intercept The estimated ADG in this study was low ( $53.4 \pm 14.1$  g/day) and with a large standard error, showing wide differences in response to treatment effects among animals. The growth rates were lower than in a 90 d performance study (plus a 14 day prior adjustment period) where weaned Boer goats feeding on chopped orchard grass (*Dactylis gromelata* L.) hay were supplemented with whole cotton seed at 0, 8, 16, 24% with corresponding ADG (g/day) of 99.2, 88.0, 82.8 and 67.3 (Luginbuhl et al., 2000). These growth rates were, however, still lower than the 168 g/day of weaned Boer goats grazing alfalfa (Luginbuhl, unpublished data as reported in Luginbuhl et al., 2000).

Goats are naturally browsers and require considerable time to get accustomed to feeding under confinement. Additionally, there was wide fluctuation in the quality of the basal grass hay which was bought from the suppliers due to the drought situation when the trial was conducted, which may have negatively impacted on the intake and digestibility. Moreover, the goats used in the trial were accustomed to feeding freely on extensive rangeland. Similarly, Luginbuhl et al.

(2000) expressed potential detrimental effects of individual housing on intake and thus performance. Confined goats ate more whole foliage hanging from the wall of a pen or tied in a trough compared to chopped foliage offered in a trough or with stripped leaves fed in a trough along with tied twigs (Van et al., 2005). It was suggested that a tridimensional arrangement by hanging feed on the wall or in a trough, allowed goats to feed from different angles, which was better suited to higher intake. Goetsch et al. (2010) concluded that numerous interactions exist between the behavior and nutrition of goats, which can affect the level and efficiencies of production.

A single measure of a dependent variable may not capture all the variation and efficiently use all available data, especially where repeated measurements are taken on individuals, hence the decision to model the weekly weights taken over the experimental period. The best covariance structure for weekly weights of Boer goats based on the BIC (Littell et al., 1998) was unstructured (UN), although the BIC from other structures (heterogeneous auto-regressive order 1 plus random effects [ARH(1) + RE], ante-dependence [ANTE(1)]) were similar. Examination of the variances across the different periods shows that there is no specific tendency for them to increase or decrease. The largest variance (week 1 = 17.0162) is 2 times the smallest variance (week 8 = 8.689), which does not indicate wide differences in variances. The covariances also tend to fluctuate, showing no specific pattern. These variations in the variances and covariances may reflect changes in the quality of the basal grass hay that was fed, among other environmental factors.

The results from the repeated measures analysis of weekly body weights nonetheless indicated that treatment, birth type, week, sex and sex x birth type interactions were influential (P



< 0.05). *Lablab purpureus* resulted in heavier weights at the 18% inclusion level and this can be attributed to the higher VFI with this legume compared to others, hence availing energy sources for the growth. *Vigna lobatifolia* tended ( $P = 0.0728$ ) to give better performance ( $P < 0.05$ ) than Lucerne at the 18% inclusion level, this was inspite of similar ( $P > 0.05$ ) VFI of Lucerne and *V. lobatifolia*; and does suggest more efficient utilization of *V. lobatifolia*. Similarly, *V. lobatifolia* gave similar performance ( $P > 0.05$ ) to Lucerne and was superior to lablab at the 31% inclusion level. This is a surprising result given that both Lucerne and lablab are improved legumes which have been selected for high nutritive value. It also suggests that digestion trials need to be taken to further evaluate this potentially useful legume in comparison to Lucerne and lablab to elucidate why it is apparently nutritionally similar, if not superior to them.

At the 18% legume inclusion level, VFI was higher under Lucerne than *V. lobatifolia*, but resultant body weights were better under the *V. lobatifolia*. It could suggest that *V. lobatifolia* is less degraded in the rumen in comparison to Lucerne, hence yielding more bypass protein which is digested and absorbed in the small intestine. Low N utilization efficiency has been reported for alfalfa silage (Broderick et al., 2000; Dewhurst et al., 2003) and this could be due to its high degradability in the rumen. Inefficient N utilization was reflected in the cows on alfalfa silages having the highest plasma urea concentrations compared to those on red clover, white clover, rye grass silages or mixtures of rye grass and white or red clover silages (Dewhurst et al., 2003).

There was a clear advantage of supplementation at 18% over 31% ( $P = 0.0023$ ) for *L. purpureus*, which may be indicative of crude protein wastage at the higher level of supplementation. Indeed most of the legumes have their protein in soluble form or is ruminal

degradable (Broderick et al., 1995), hence any excess ammonia over what can be captured for microbial protein synthesis goes to waste and may impose a metabolic cost to eliminate it (Coblentz and Grabbert, 2013). Tyrrell et al. (1975) having reviewed calorimetry studies reported a reduction of Metabolizable Energy (ME) and Net Energy (NE) of rations when digestible protein consumption exceeded requirements. It has been suggested that the effect of excess protein on the efficiency of energy utilization is linked to amino acid catabolism, rather than urea synthesis *per se* (Reynolds, 2006). The similar average body weights ( $P > 0.05$ ) for the combined groups of goats at 18% versus 31% legume inclusion despite overall higher VFI at 31% is puzzling, but may be due insufficient fermentable energy in the rumen. On the contrary, higher weight gains were realized within the treatments (i.e. OB18 vs. OB31; VL18 vs. VL31; LC18 vs. LC31) at the higher legume inclusion level. This we believe is due to the higher VFI that was possible, availing extra energy for growth.

In a study with lactating Holstein cows, 16.5% of dietary CP appeared to be sufficient for maximal ruminal outflow of total bacterial nonammonia N (NAN) and total NAN (Colmenero and Broderick, 2006). Indeed it was recommended that Intake of N by high producing cows that consumed sufficient energy and other nutrients for their requirements could be decreased to 600 to 650g/d (~16.5% CP on DM basis) provided the source of RDP and RUP were properly matched with the source and amount of carbohydrate in the diet. This feeding approach should be sufficient to optimize cow productivity throughout the whole lactation and improve efficiency of feed N conversion to milk N (Ipharraguerre and Clark, 2005). In ruminants, weight gains were optimised at 20% dry matter intake supplementation with dried *Leucaena leucocephala* leaves (Jones 1994; Rubanza et al., 2007). Given that most forage legumes tend to be high in RDP, there may have been insufficient quantities of protein passing to the lower intestinal tract, hence

reducing available amino acids to the goats for tissue accretion. In this study, it is also plausible that there was a shortage of fermentable energy because of the poor quality of the basal grass hay during some stages of the trial, moreover no starch sources (e.g. maize grain) were provided to the goats, possibly leading to excretion of excess N via the urea cycle (McDonald et al., 2010).

Weekly variations in weights of Boer goats are explained by fluctuations in quality of basal grass hay on offer. Indeed body weights generally tended to increase in weeks 3 to 6 of the trial and then declined in weeks 7 to 10. When goats were moved from the range to the pens, they lost weight in the first two weeks as they adjusted to the confinement conditions to which they were not used. The goats started gaining weight under confinement feeding in the subsequent four weeks, but unfortunately the quality of the basal grass hay on offer in weeks 6 to 9 declined coupled with a possible increase in temperature, which resulted in a corresponding reduction in voluntary feed intake and body weights. The regression coefficient of ADG on initial body weight was negative which may be explained by the sigmoid growth curve. As animals mature their growth rate slows down, hence it is plausible that the goats with heavier initial body weight were older than the lighter ones, hence they were expected to have slower growth rates. Goats born as singles may have benefitted from a carryover effect of heavier body weights due to greater milk consumption compared to twins.

Voluntary feed intake on metabolic body weight basis (VFIMBW) varied from  $62.8 \pm 5.5$  (control treatment in week 9) to  $134.6 \pm 6.4$  (*L. purpureus* at 31% inclusion [LP31] in week 4) g DM/kg  $W^{0.75}$ /day; the average was  $97.6 \pm 1.6$ . This is much higher than the  $71.0 \pm 10.5$  to  $80.2 \pm 3.8$  g DM/kg  $W^{0.75}$ /day reported for Tswana goats on a basal diet of buffel grass supplemented with different locally available browses (Aganga and Monyatsiwa, 1999). Conversely, the

VFIMBW (g DM/kg  $W^{0.75}$ /day) was lower than in six month old West African Dwarf (WAD) goats that were on a basal grass hay of *Panicum maximum* supplemented with forage legumes and attained intakes varying from  $108.2 \pm 4.1$  (*P. maximum*) to  $138.7 \pm 4.1$  (*P. maximum* + *Stylosanthes guianensis*) (Ajayi et al., 2008). The percentage increment in VFIMBW at the 31% legume (*L. purpureus*, *O. burchellii* and *V. lobatifolia*) inclusion level over the control group in our study (5%), was higher than that reported for *L. purpureus* (30%) in Ajayi et al. (2008) who used grass: legume mixtures of 60:40.

Metabolizable energy for maintenance usually does not increase in direct proportion to body weight, hence ME/kg  $W^{0.75}$  will be smaller for heavier animals than light ones (Holmes et al., 2007). Also, compared to sheep and cattle, goats frequently have higher ad libitum feed intake relative to body weight (AFRC, 1998). Intake per unit of metabolic weight tends to be high in lean animals and is manifested in animals showing compensatory growth after a period of feed restriction and also ruminants with chronic food shortage (McDonald et al., 2010). The estimated ADG from the intercept was low ( $53.4 \pm 14.1$  g/day) which indicates poor rates of gain for Boer goats at this stage of maturity, hence they were generally in lean condition which may have contributed to the high voluntary feed intake on a metabolic body weight basis. The poor quality of the basal grass hay may have led to high substitution rates with the legumes so as to meet the goats' nutrient requirements, hence contributing to the high intakes per unit of metabolic body weight.

Examination of the variances in VFIMBW excluding the two week adjustment period (weeks 3 to 11), indicates that there is not much evidence to suggest that variances among the treatments are different, with the largest variance only about 4.5 times the smallest one. This

would suggest that VFIMBW may be a more robust tool for comparing intakes under different treatments, especially when there are disturbing factors in experimentation, like the drastic fluctuations in basal grass quality experienced in this study. The weekly fluctuations in weights of Boer goats were partly due to growth but this was disturbed by changes in the environment (feed quality, temperatures, housing, management) to which animals were subjected. Modeling of weekly weights as repeated measures using Proc Mixed (SAS, 2008) enabled disentanglement of confounding effects due to fluctuations in basal grass hay and other environmental variables and permitted estimation of effects due to treatment.

## 5.5 Conclusions

Legume supplementation of basal grass hay increased VFI with the increase at 31% exceeding that at 18% inclusion. At the 18% inclusion level, VFI was greatest with lablab and lucerne, followed by *V. lobatifolia* and *O. burchellii*. These results are indicative of the high nutritive value of the indigenous legume *V. lobatifolia* which is comparable to that of lucerne and *L. purpureus*, but needs to be assayed for antinutrients. *Otoptera burchellii* is also promising, although ranking lower than *V. lobatifolia*. Although supplementation at the 31% inclusion level gave the greatest VFI, there were no differences in ADG of combined control and supplemented goats. ADG was lower than in other studies, possibly due to lack of adaptation of goats to individual housing, inadequate fermentable energy supply and fluctuations in the quality of basal grass hay offered.

Studies are needed to optimize the inclusion levels of indigenous forage legumes coupled with a readily available fermentable energy source (e.g. starch) in ruminant diets. Supplementation with the legumes lucerne, lablab, *V. lobatifolia* and *O. burchellii* at 18%

inclusion level, positively impacted weights in weaned Boer goats; among the native legumes, *V. lobatifolia* stands out and warrants further study. Supplementation of Boer goats with legume included at 31% in the diet, may be wasteful and indeed incur an energy cost to the animal in getting rid of the excess wastes of Nitrogen metabolism.

## CHAPTER 6

### 6 General Discussion

#### 6.1 Fodder production potential of native legumes

Sustainability of agricultural production in many parts of the world is threatened by many factors including climate variability, soil degradation and salinity (Lambers, 2003; Howden et al., 2008). Dealing with these challenges, requires production strategies that are better suited to the local conditions. The use of native legumes in grazing systems constitutes one such adaptation strategy that could improve the resilience and sustainability of production (Bennett et al., 2011; O' Keeffe et al., 2015). Native legume populations in rangeland have declined because of changes in land use, selective grazing, extensive use of herbicides for broad weed and bush control and bush encroachment (Muir et al., 2011). This study was part of a wider project investigating potential utility of indigenous legumes of Namibia in feeding livestock, hence it is important to put this in a wider context by examining desirable attributes of legumes in animal feeding systems. Lucerne is the predominant forage legume used for feeding livestock in Namibia, but native perennial legumes may provide potential alternatives.

Certain legumes belonging to the genus *Arachis* (forage peanuts) are commonly used for feeding animals in Brazil (Valls and Pizarro, 1994) because of their versatility as they can be grazed in pastures, used as fresh cut forages, hay or silage (Ferreira et al., 2012). These legumes also have adequate productivity, are persistent in combination with certain grasses and are of high nutritive value (Lascano, 1994). Dry matter productivity has ranged from 7 to 16 tons/ha<sup>1</sup>year and the ability to form persistent consortiums with runner habit growth grasses like those from the genus *Brachiaria* and *Panicum* have been reported (Andrade et al., 2006; Valentim et al., 2001). Native legumes in Namibia need to be identified and evaluated on agronomic aspects,

growth habits, nutritive value and optimal management to enhance animal performance sustainably.

The legumes investigated in this study differ in growth habits: *O. burchellii* forms runners, although it has been described as a climbing shrub ((Moteetee and Van Wyk, 2011); *Cullen tomentosum* is semi-erect; *Medicago laciniata* is prostrate; *Meulobium candicans*, Lucerne (*Medicago sativa*) and *Crotalaria argyraea*, *Crotalaria podocarpa* and *Crotalaria heidmannii* are erect; *Lablab purpureus* subsp. *purpureus* is a semi-erect bushy perennial; and *Vigna lobatifolia*, *Rhynchosia totta* (Thunb.) DC. var. *totta*, *Lablab purpureus* subsp. *uncinatus* var. *rhomboideus* (uncultivated lablab) are trailing. The different growth habits imply the legume species could be tailored for different production purposes for example grazing or as fodder for cut-and-carry systems or used as hays and silages. Growth habit will also be important in choice of compatible grasses with which grass-legume mixtures can be formed. Bunch grasses for example buffel grass (*Cenchrus ciliaris*), love grasses (*Eragrostis* spp.) tend to predominate in areas receiving < 800 mm of annual rainfall and in well drained soils (Diggs et al., 1999; Moser et al., 2004). Bunch grasses are advantageous in grass-legume mixtures because they allow forb seedling establishment in the space between plants (Springer et al., 2001). Biomass production (tons DM/ha) in legumes included in this project varied from  $1.5 \pm 0.4$  (*V. lobatifolia*) to  $8.9 \pm 0.3$  (*L. purpureus* subsp. *purpureus*) (Kaholongo, 2016), but possibly could be optimized with adequate irrigation and fertilizer application under intensive production. Estimates on productivity for some legumes were, however, not obtained due to time constraints and lack of seed or other planting materials.

The growth habits of the legumes determine how best they could be utilized for feeding ruminants. *Vigna lobatifolia* has a trailing habit and can stretch over 5 m and was easily



harvested by hand pulling and cutting at the base using a knife. It could also be grazed in the field. *O. burchellii* forms runners from which adventitious roots develop at nodes; it also forms tough stems making it relatively difficult to harvest. *O. burchellii* may be best utilized for grazing. *Rhynchosia fleckii* was observed to be frost sensitive and was one of the first legumes to shed its leaves when temperatures dropped in mid April in central Namibia; grazing is the most suitable mode of utilization for this legume. *Rhynchosia totta* (Thunb.) DC. var. *totta* is a trailing species and is best utilized under grazing but could also be harvested for fodder. *Lablab purpureus* is commonly utilized as fresh forage in cut –and-carry systems, although fodder hays are also produced on large scale (Adebisi and Bosch, 2004).

The demonstration that most of the legumes (with the exception of *Ptychlobium biflorum*) could be propagated by seed or plantlets (Kaholongo et al., 2016) should encourage efforts to optimize growth conditions of these native legumes so that seeds are available for trial fodder production, once the legumes have been assayed for antinutrients. Seed multiplication is a crucial step in tapping the potential of native legumes as lack of seed constrains wide use of this resource. Fodder production potential is also dependent on their persistence (Chapman et al., 2015) which is influenced by their seed production, food storage reserves and grazing pressure. Legumes like *V. lobatifolia* have storage tubers which may provide water and other nutrients that enable them to persist through drought years (Sprent et al., 2010), contributing to their adaptability in semi-arid areas. Under cultivation, seed production was greatly enhanced for *V. lobatifolia*, but seed collection had to be frequent because pods dehisced immediately after ripening (Kaholongo, L. T. pers. Comm., 2016). *Crotalaria podocarpa* has been identified as an encroacher species in Namibia's escarpment region which has reduced biomass production and vigour of perennial grasses and permanently damaged grass tussocks (Fischer et al., 2015).

Native legumes are an important aspect of sustainability because some species are adapted to low soil nutrient availability, have tolerance for saline soils and when grown under soils with limited moisture may have better survivability compared to exotics and are a potential valuable feed resource for livestock (Aswathappa et al., 1987; Denton et al., 2006; Dear et al., 2007; Suriyagoda et al., 2010; Bennet et al., 2011). Research on undomesticated *Cullen* species in Australia relative to Lucerne showed their potential as pasture for arid environments, due to their root plasticity which enabled them to access P and moisture efficiently (Suriyagoda et al., 2010). Soils in southern Africa are generally deficient in phosphorous; salinity is a concern in some parts of Namibia and droughts are recurrent, hence the need to exploit forage legumes native to Namibia for enhancing livestock production.

Numerous studies have demonstrated the feeding value of legumes; in particular studies have shown the superiority of legume silages over grass silages (e.g. Thomas et al., 1985; Hoffman et al., 1998). Notwithstanding, the area under forage legumes has been on the decline for several reasons including the relatively low cost of Nitrogen fertilizer, and difficulties with the agronomy and ensiling of legumes (Dewhurst et al., 2003). In semi-arid regions, low soil moisture is a major hindrance to establishment of forage legumes and their persistence (Peoples and Baldock, 2001). Legume growth is also constrained by low P availability, soil acidity or sodicity. These adverse influences can be ameliorated with fertilizer, lime or gypsum if they are localized in the top soil (Peoples and Baldock, 2001). Efforts to characterize native legumes may identify species of legumes better adapted to the harsh environments in semi-arid regions of the world, hence the importance of this research. Evaluation of forages, entails high costs and therefore it is important that preliminary screens be done rapidly to identify those native legumes with greatest potential so they can undergo more detailed analyses. This study aimed at

contributing to such efforts by examining the plant morphology, doing chemical analyses, carrying out palatability and voluntary feed intake trials.

## **6.2 Nutritive value and palatability of native legumes in comparison to lucerne and lablab**

In preliminary selection of which legumes to include in the agronomic trials (Kaholongu, 2016), emphasis was on potential biomass production, growth habits (runner, prostrate, trailer, erect), absence of physical (thorns, waxiness, hairiness) or chemical (ordours) deterrents. Presence of antinutrients is also an important aspect, although these were not assayed. Potential utility of forage legumes in ruminant feeding will be determined by nutritive value, persistence under grazing and presence of antinutrients.

As one of the key steps in tapping the potential of native forage legumes, it is important to determine their chemical composition and this was done in experiment 1. Analysis of the chemical composition was carried out on 14 forage legumes and with the exception of *M. candidans* (CP =  $12.3 \pm 2.1\%$ ), the CP content was greater than 15%, indicating their potential use as supplements for ruminants grazing grass pastures. All the forage legumes had NDF concentration within acceptable ranges; Van Soest (1994) suggested that NDF concentration on DM basis lower than 550-600 g/kg DM would not limit intake. The CP availability and its efficiency of utilization will, however, be dependent on plant secondary compounds such as condensed tannins which influence adhesion, colonization and enzymatic activity of the rumen microbial system and hence degradation of different dietary fractions (Guimaraes-Beleen et al., 2006; Yifei et al., 2009). Concentrations of plant cell wall contents are negatively correlated with palatability, voluntary DM intake and potential DM degradability of forages (Kaitho et al., 1998;

Larbi et al., 1998), hence observed variation among forage legumes have implications for optimizing ruminant feeding systems.

Ferreira et al. (2012) in evaluation of forage peanuts in Brazil for feeding livestock, used chemical composition and *in vitro* DM digestion kinetics. In categorizing legumes into groups using multivariate analyses, the variable kd (degradation rates), CP and DP48 (potential DM degradation at 48 h) were found to have more discriminating power to compose groups (Ferreira et al., 2012). These same variables were highly correlated with the nutritional value of feedstuffs and animal performance (Barnes et al., 2007). This study used chemical analyses, palatability and voluntary feed intake trials to assess the feed value of the native legumes. The ME (MJ/kg DM) varied from 8.7 (*L. purpureus* subsp. *purpureus*) to 10.7 (*C. heidmannii*), indicating they could contribute to improved production in ruminants. In this study *in vitro* digestion kinetics (kd and DP48) were not estimated and it would be useful in future studies to estimate these parameters when screening forage legumes so as to evaluate further those with greatest potential. Although the rate of gas production in the *in vitro* techniques is correlated to feed degradation, it does not directly correspond to the extent of feed degradation (Mohamed and Chaudhry, 2008). Feeds with a low gas production rate may indeed have higher *in vivo* digestibility than would be suggested by the *in vitro* methods and this may be due to post-ruminal activities that only occur due to *in vivo* digestion of a feed (Menke et al., 1979). Hence results from the *in vitro* digestion need to be collaborated further by *in vivo* digestion and voluntary feed intake studies.

Relative palatability was carried out on a selected sample of legumes (*Clotalaria argyraea*, *C. podocarpa*, *O. burchellii*, *V. lobatifolia* and Lucerne) primarily based on availability because the native legumes could only be sourced from the wild. The proportionate consumption of *C. argyraea* did not differ from that of *C. podocarpa*, possibly because they are

similar in chemical composition and may have similar antinutrients. Unlike the *Crotalaria* species whose consumption was low and fluctuated in the first 5 days, the forage legumes *O. burchellii*, *V. lobatifolia* were consumed in proportionately high amounts comparable to Lucerne, throughout the 10 day trial period. The relatively high palatability may indicate high nutritive value and low concentrations or absence of antinutrients in *O. burchellii* and *V. lobatifolia*. But it has also been suggested that animals sample novel feeds cautiously (Provenza, 1995) and given that the *Crotalaria* species were obtained from a different agro-ecological zone, this may have contributed to the lower initial consumption. Overall, the results indicate that all the forage legumes included in the palatability trial may not pose intake problems and could be evaluated in conventional feed trials, but the presence of pyrrolizidine alkaloids and flavonoid glycosides in the aerial parts (Wanjala and Majinda, 1999) in *C. podocarpa*, suggests it should not be evaluated further; *C. argyrea* should, however, be analysed for antinutrients.

Foods that are digested rapidly promote high intakes (McDonald et al., 2010), and the more rapidly the digestive tract is emptied, the more space is made available for the next meal. The NDF concentration is a primary determinant of digestibility; a negative relationship exists between the NDF content of foods and the rate at which they are digested (McDonald et al., 2010). Voluntary intake is one of the best indicators of the nutritive value of a feed (Minson, 1990). In the feeding trial (experiment 3), voluntary feed intake was higher at the 30% legume inclusion level than at the 15% level, which was higher than at 0% level.

The least squares means for the NDF (%) of the basal grass hay varied from  $56.9 \pm 3.3$  (predominantly *Antephora pubences*) to  $62.5 \pm 4.0$  (predominantly *Stipagrostis uniplumis*). The least squares means for NDF concentration (%) of the legumes used in the feeding trial varied from  $36.0 \pm 1.8$  (lucerne) to  $44.4 \pm 1.9$  (*V. lobatifolia*) which was much lower than for the

grasses, hence digestion was rapid which promoted higher feed intake. The increased DM intake under supplementation, may have led to increase in passage rate through the rumen, decreasing the extent of DM digestion due to a lower exposure time of feed particles to the enzymatic action of rumen bacteria (Djouvinov and Todorov, 1994; Shem et al., 1995; Illius et al., 2002). The legumes *L. purpureus*, *O. burchellii* and *V. lobatifolia* promoted intakes comparable to Lucerne and hence provide an attractive alternative for supplementation of grass hays. In general grasses have higher NDF concentration than legumes, hence at the same digestibility 20% more legumes than grasses will be consumed. Also lignification in legumes is restricted to vascular bundles whereas in grasses it is more widely distributed and has a greater inhibitory effect on rate of digestion (McDonald et al., 2010). The differences in VFI due to the legume supplement and level signal possible differences in degradation characteristics, which will also influence protein use efficiency.

Taking into account the results from the chemical analyses and palatability trials, the legumes *V. lobatifolia* and *O. burchellii* appear to have good potential for feeding livestock, but they need to be assayed for antinutrients. In addition *L. purpureus* subsp. *purpureus* is an established fodder commercially grown in many countries (Adebisi and Bosch, 2004) and Kaholongu (2016) demonstrated that high biomass production was also possible under Namibian semi-arid environments, hence it provides an immediate alternative option for fodder production. Studies are needed to determine the palatability, feed intake and antinutrients in the native legumes *P. biflorum*, *C. argyraea*, *R. fleckii* and *R. totta* (Thunb.) DC. var. *totta*. Additionally, search should continue to identify more native legume species and cultivars in Namibia for evaluation as animal feed resource.

### 6.3 Effect of legume supplementation on voluntary feed intake and body weights

Supplementation of lambs with legume hays (annual and perennial peanut, cow pea, pigeon pea, soybean) increased DM and OM intake, although only supplementation with annual and perennial peanut hay also increased DM and OM digestibility (Foster et al., 2009). In a review of studies on supplementation of high producing cows (> 25 kg/day in early lactation in that study) on pasture, Bargo et al. (2003) concluded that protein supplementation increased digestibility of OM and NDF and N intake and flows of NAN and NANMN (nonammonia, nonmicrobial nitrogen) without affecting the flow of microbial N. Supplementation with CP has been reported to increase DM, OM and NDF digestibility (DelCurto et al., 1990; Horney et al., 1996; Bohnert et al., 2002). It is thought this is due to improved nitrogen availability by the ruminal microflora which increases ruminal fibre digestion (Campling et al., 1962; Petersen, 1987).

Legume supplementation does not usually increase NDF digestibility (Haddad, 2000; Mupangwa, 2000; Foster et al., 2009), in part because legumes have more lignin than grasses (Wilson, 1994). In this study, supplementation with legumes increased DM and OM intake, but the effect on digestibility was not established. Among the legumes investigated lowest NDF concentration (%) were found in *C. argyraea* ( $36.2 \pm 3.8$ ), *C. heidmanii* ( $30.3 \pm 3.8$ ), *L. purpureus* ( $39.2 \pm 3.1$ ), *M. sativa* ( $36.0 \pm 1.8$ ) and *P. biflorum* ( $34.1 \pm 2.4$ ) which were much lower than in the forage legumes reported by Foster et al. (2009). Protein supplementation increases forage intake and this is associated with increases in rates of passage, rumen fill or both (McCullum and Horn, 1990). It is apparent that improved forage digestion contributes to increased intake and passage rates in some cases. Supplementation in this study may have increased microbial activity, reducing rumen fill and increased the passage rate hence leading to

increased intakes. Supplementation with forage legumes may also help in buffering rumen pH resulting in better rumen conditions for fibre digestion (Nousiainen et al., 2009).

In a comparison of the in situ degradation of alfalfa, birdsfoot trefoil, red clover at various stages of maturity (late vegetative, late bud and mid bloom) and brome grass, orchard grass, rye grass timothy and quack grass at second node, boot and full inflorescence, the CP content declined and NDF content increased with maturity, but those changes were more pronounced with grasses than in the legumes (Hoffman et al., 1993). With the exception of rye grass, legumes tended to have a larger effective degradability (ED) of DM than grasses at all maturity stages; rye grass had ED of DM similar to legumes. In that study legume species did not differ in ED of CP. Legumes generally had lower degraded NDF than grasses; rye grass had the highest ruminally degraded NDF of all species at all stages of maturity (Hoffman et al., 1993).

The higher intakes observed in our study with supplementation corresponding to the increased consumption of legumes in preference to the basal grass hay, may be due to the higher effective degradability of legume DM, because of the lower NDF concentration (Foster et al., 2011) which reduced rumen fill. None the less, this was wasteful as it possibly led to excessive ammonia production in the rumen that had to be excreted in urine because of insufficient availability of fermentable energy. This was more pronounced at 30% legume inclusion level, which despite resulting in significantly higher VFI, led to lower body weights than at 15% inclusion. In a meta-analysis, Bargo et al. (2003) found that the most consistent effect of concentrate supplementation on rumen fermentation was a reduction in ruminal  $\text{NH}_3\text{-N}$  concentration. Rumen  $\text{NH}_3\text{-N}$  concentrate reduction they reasoned was possibly linked to a higher capture of  $\text{NH}_3\text{-N}$  from the highly ruminally degradable CP of pasture (Van Vuuren et al.,



1986; Bargo et al., 2002a), but also to a reduction in total CP intake because energy supplements are usually lower in CP than pasture (Berzaghi et al., 1996; Garcia et al., 2000).

The high CP levels due to supplementation in this study stimulated microbial activity possibly leading to high digestibility and reduced rumen fill, hence increased intake. Nonetheless, because there may not have been sufficient fermentable energy available to the rumen microbes, the increased intakes did not translate into higher growth rates for the group at 30% over the one at 15% supplementation level. Indeed at the 15% supplementation level, only *V. lobatifolia* was clearly superior ( $P = 0.0014$ ) to the control, with Lucerne only showing a trend ( $P = 0.0562$ ). Given that the CP content in the basal grass hay was below the minimum threshold of 7% (Milford and Minson, 1965), it would be expected that supplementation would be beneficial. It is plausible, however, there was inadequate supply of fermentable energy and this was compounded by the poor adaptation of the goats to the confinement.

The different legume supplements and their inclusion level affected VFI. NDF is thought to be linked to the fill effects of feed (Mertens, 1987, 1994). The insoluble fraction of the diet, which can occupy space in the GIT is represented by NDF (Van Soest, 1994). NDF includes indigestible NDF (iNDF) and potentially digestible NDF (pdNDF) and these fractions have different ruminal kinetics and hence passage rates (Stensig and Robinson, 1997). The fill effects of the fibre will, however, depend on a number of aspects including the dimensions of the indigestible and potentially degradable fractions and the rate of removal from the rumen (Waldo et al., 1972; Detmann et al., 2010).

The rumen fill (RF) expresses the steady state fill per unit of fibre intake and gives an indication of the capacity of fibre to occupy space in the reticulorumen compartment (Detmann et al., 2014). The rate (kd) and extent of digestion (the ratio of pdNDF to iNDF) will determine

RF and hence the fibre digestibility. Emphatically, RF is a characteristic of the fibre itself and does not depend on the dietary fibre content (Detmann et al., 2014). Hence it would appear, *V. lobatifolia* and *O. burchellii* which despite having higher ( $P < 0.05$ ) NDF were consumed in similar ( $P > 0.05$ ) quantities to Lucerne or higher ( $P < 0.05$ ) quantities than *L. purpureus* subsp. *purpureus* at 30% inclusion level, may differ in rate and extent of fibre digestion.

Ruiz et al. (1995) reported improved milk production and DMI when diets formulated to similar NDF concentrations, contained fibre of higher quality. Hence in addition to NDF concentration, dietary fibre digestibility may be important in influencing DMI and milk production. Fibre digestibility has important implications for ration formulation and prediction of DMI, bearing in mind the forage source (Ruiz et al., 1995). Differences observed in intake among different forage legume supplements may also be due to antinutrients and (or) physically effective fibre. Dry matter intake is sensitive to dietary physically effective NDF (peNDF); as peNDF decreases, passage rate and DMI increases (Teimouri Yansari et al., 2004; Zebeli et al., 2008); on the other hand, excessive peNDF may reduce voluntary feed intake due to the physical limitations of rumen fill (Allen, 2000; Zebeli et al., 2010).

Ruminants need both rumen undegradable protein (RUP) and rumen degradable protein (RDP) and hence the need for balancing these in the diet. Inadequate N availability to rumen microbes may adversely affect fibre digestibility (McAllan and Griffith, 1987). Inadequate microbial activity implies that fibre has a greater filling-effect and thus feed intake can be reduced (Ciszuk and Lindberg, 1988). The basal grass hay had a CP of  $5.6 \pm 1.4$  to  $6.0 \pm .8$ , which is below the minimum threshold of 7% (Milford and Minson, 1965). This explains why the intake of the basal diet in the feeding trial was the lowest due to insufficient microbial activity stemming from inadequate CP content. Inclusion of legumes boosted feed intake,

although at the higher inclusion level (30%), the increase was mainly due to the legume consumption. While grass leaves have parallel venation, legume leaves have reticulate venation, which results in less NDF concentration and greater rates of particle passage in legumes (Frame, 2005).

Most of the protein in legumes is highly rumen degradable (Broderick, 1995). Once the minimum threshold of N for ruminal microbial functioning has been reached, no additive benefits accrue to supply of more RDP (Madsen and Hvelplund, 1988). Analysis of body weights indicated supplementation at 15% legume inclusion level was of greater benefit than at 30%; the higher level might indeed have incurred a metabolic cost in eliminating excess ammonia. Excess RDP is converted to ammonia in the rumen and diffuses to the blood. Similarly, once the UDP requirement has been met and there are not any limiting amino acids, any excess UDP only serves as an additional energy source (Mohammed and Chaudhry, 2008). Dewhurst et al. (2003) in experiments with dairy cows consuming legume silages, observed a general decline in N use efficiency with increasing legume content in the diet, which was linked to increasing N intake. It was inferred that balancing red clover N with some other dietary component was of greater benefit than the inherent attributes of red clover (Dewhurst et al., 2003). Future research should therefore constitute forage mixtures and protein and energy supplements to optimize N efficiency with diets based on native forage legumes.

Higher intakes of legume silages compared to grass silages resulted in higher yields of milk, milk fat, milk protein and milk lactose in dairy cows (Dewhurst et al., 2003). Alleviation of a ruminal nitrogen deficiency is generally considered one of the main mechanisms protein supplements increase intake and digestion of low quality forages (McCollum and Horn, 1990). This was evident by the increased VFI under different supplementation levels such that 30% >

15% > 0%. Inadequate fermentable energy levels (De Visser et al., 1991; Windschitl and Schingoethe, 1984; Petit and Tremblay, 1995), coupled with insufficient RUDP may, however, have contributed to inefficient N utilization and future studies should investigate this aspect.

#### **6.4 Legume contribution to diversity and its importance in rangelands**

A highly diversified grassland plant community is associated with increased primary (plant) production (Sanderson et al., 2005), greater stability in response to disturbance (Minns et al., 2001) and reduced weed pressure (Tracy and Sanderson, 2004). Ruminant livestock production in Namibian rangelands is a low-input system that relies on complex species mixtures to produce forage. Research with forage mixtures in clipped plots indicated increased herbage yield with complex as opposed to simple mixtures of forages (Deak et al., 2004; Tracy and Sanderson, 2004). Soder et al. (2006) investigated altering complex forage mixtures in pastures and their results indicated this did not affect dry matter intake, milk production or blood metabolite profiles of lactating cows.

Indigenous forage legumes investigated in this study provide opportunity to increase diversity in rangeland plant species thereby potentially increasing primary production, rangeland resilience and potentially animal performance. A diversity of plant species also permits animals to select, which may optimize nutrient usage and avoid adverse effects (Maughan et al., 2014) from antinutrients like saponins in lucerne when fed in excess. Goats consumed the five (5) species (*C. argyrea*, *C. podocarpa*, *V. lobatifolia*, *O. burchelli* and *M. sativa*) used in the palatability trial almost completely after a five (5) day adjustment period, which is indicative of benign postingestive effects, although long-term feeding trials may be needed to identify any adverse effects of prolonged feeding with specific legume supplements on voluntary feed intake, health, growth rate, meat quality, milk production and reproductive performance.

Grazing land management should aim at giving animals the opportunity to select from a wide range of forages, while allowing high rates of production per hectare (Chapman et al., 2007). The availability of feeding materials restricted the scope for palatability trials, but indications were that all four legumes (*C. argyraea*, *L. purpureus*, *O. burchellii* and *V. lobatifolia*) tested were equally comparable in palatability to *M. sativa*. The differences in nutrient and antinutrient composition provide room for selection by the ruminant animal to match its requirements, while minimizing the adverse effects. The forage legumes differ in growth habits and nutrient requirements; possibly they also differ in drought tolerance, yield potential and persistence, hence it is conceivable the different legumes showing potential can be suited to different agro-ecological zones and find applications under different settings (pasture, cut-and-carry, hay or silage production). Research is needed to test different consortia of legumes in combination with common grasses to determine those with potential for high rates of production per hectare.

Although we could not obtain information on *V. lobatifolia* in the literature, numerous species of *Vigna* (e.g. *V. unguiculata*, *V. aconitifolia*, *V. adenantha*, *V. angularis*, *V. radiata* and *V. umbellata*) are used for forage production (Brink and Belay, 2006). *V. unguiculata* can be incorporated in forage production programs in Namibia because it is drought tolerant and is already in use for forage in other countries. For instance, Foster et al. (2011) investigated the nutritive value and ensiling characteristics of annual and perennial peanut and cow pea silages and found that they could be important energy and protein sources for dairy cows fed warm-season grasses due to their relatively high *in vitro* digestibility, CP concentrations and aerobic stability. Given the high palatability, voluntary feed intake, high CP content and acceptable NDF concentration, reasonable biomass production potential and being a perennial, there is need to

investigate further *V. lobatifolia* for antinutrients before it can be recommended as a forage crop. *O. burchellii* is a deep rooted perennial, had high CP ( $22.2 \pm 1.1\%$ ); it also led to better body weights at 30% at supplementation level than *L. purpureus* subsp. *purpureus* and Lucerne; and can be vegetatively propagated because it readily forms adventitious roots. It warrants investigation for degradation parameters and antinutrients.

A key limitation in the utilization of high-quality temperate legumes (e.g. lucerne, white clover [*Trifolium repens*]) is because they undergo extensive degradation in the rumen (Julier et al., 2003). The animal derives maximum benefit when a large proportion of the proteins go to the small intestine where they are degraded into amino acids and absorbed. Tannin concentrations above 5% DM in forages, protect proteins from extensive degradation in the rumen (Min et al., 2003). Investigations on variability in crude protein degradability in the rumen may identify cultivars that undergo less extensive degradation; alternatively biotechnological manipulations may be possible to alter tannin concentration or its monomeric composition (Julier et al., 2003). It should, however, be noted that escape protein content of forage legumes may have an undesirable correlation with other forage quality parameters (e.g. decreased protein content, decreased in situ dry matter degradation) (Griffin et al., 1994; Coulman et al., 2000).

In the voluntary intake trial, *V. lobatifolia* was the only legume that resulted in body weights that were higher than the control, which may indicate less degradability in the rumen compared to Lucerne. Also *O. burchellii* may have different rumen degradation characteristics that led to better performance compared to Lucerne and *L. purpureus* subsp. *purpureus* at 30% inclusion level. These are indications that suggest the need to exploit the enormous diversity in the native legumes, which could be used in combination with other feed resources to enhance

animal productivity. Persistence of these legumes under grazing or different harvest regimes (e.g. every 8 or 12 weeks) warrants investigation in future studies.

### **6.5 Antinutrients in fodder legumes**

In addition to being an encroacher species, *C. podocarpa* contains pyrrolizidine alkaloids and flavonoid glycosides in the aerial parts (Wanjala and Majinda, 1999) and has been reported to be unpalatable and can even cause livestock poisoning (Botha and Penrith, 2008). *Crotalaria* species are the cause of at least three types of poisoning in livestock: (i) Crotalariosis is an acute or chronic condition that is caused by ingestion of large quantities of *Crotalaria* in a short period of time, or smaller amounts over a long period, resulting in serious liver and lung damage; *C. spartioides* is particularly toxic; *C. dura*, *C. globifera* and *C. juncea* may also cause liver damage in horses, cattle and sheep; (ii) *C. juncea* causes hair loss in sheep; and (iii) *C. burkeana* causes stywesiekte ('stiffsickness'), where there is an inflammation of the horn-forming membrane of the hoof, resulting in an abnormal gait (Steyn, 1949; Vahrmeijer, 1981; Kellerman et al., 1988; van Wyk et al., 2014).

Although in this study palatability of *C. podocarpa* after a 5-day adaptation period was similar to that of Lucerne and did not lead to any detectable adverse effects on the experimental animals, the presence of poisonous principles is a warning flag against use of this legume for fodder because it may have longer-term adverse effects on animal health that could not be discerned in this short-term trial. Palatability of *C. argyraea* was similar to Lucerne, but it should be investigated for poisonous principles. The apparent absence of adverse effects in the palatability trial may be due to smaller quantities of supplement fed (100 g on as-fed basis), the short duration of the feeding trial (10 days) or some adaptive mechanism in goats to combat the poisonous principles.

Many plants produce plant secondary metabolites (PSM) which may serve as deterrents against herbivory (Waghorn, 2008). Condensed tannins (CT) in particular are widely distributed in plants and can have positive benefits on animal nutrition and health (Waghorn, 2008). Condensed tannins at concentrations of 2 – 4% have been shown to be beneficial as they protect protein from excessive degradation in the rumen (Barry and McNabb, 1999; Min et al., 2003). Although assays for antinutrients were not done, the greater weights of animals at 31% *O. burchellii* (OB31) inclusion level compared to those at 18% (OB18) level, in sharp contrast to other legumes (lablab, *V. lobatifolia*, Lucerne) may be indicative of presence of tannins or other compounds that lead to its more efficient use.

Most of the literature abounds with antinutrients found in temperate legumes like Lucerne, clover, birdsfoot trefoil, sainfoin and *Sericea lespedezia* (*Lespedeza cuneata*) (e.g. Waghorn et al., 1994b; McAllister et al., 2005). It is important that the different native legumes in Namibia be assayed for CT and other antinutrients (e.g. saponins, alkaloids, lectins) to enable efficient and effective utilization of these feed resources for improving livestock productivity. The anti-nutritional effects of high PA concentrations in tropical forages can be reduced by drying (Waghorn and McNabb, 2003). All the forage legumes in this study were fed as hays and hence the anti-nutritive effects stemming from condensed tannins, if these exist may have been somewhat mitigated.

## **6.6 Further gaps in information to explore on native legumes**

Native forage legumes may have many benefits for grazing systems besides their value as feed resources. In Australia, O'Keeffe et al. (2015) for instance reported that seedlings of *Glycine canescens* were well adapted to low-P stress during establishment and were shown to have higher DM yields than introduced legume species such as Lucerne under low-P conditions



(Pang et al., 2010). Many soils in Namibia are deficient in P and hence native legumes found growing in specific localities, may be efficient at utilizing the limited P in such soils. Native legumes may also form symbiotic relations with bacteria, contributing to biological nitrogen fixation (BNF). Some of the legumes could potentially be used as cover crops and may be useful in restoring degraded rangelands.

Effective and efficient utilization of native legumes requires understanding of their impact on grazing systems, their sensitivity to grazing pressure and cutting frequency (O' Keeffe et al., 2015). It is also important to estimate their water use efficiency in comparison to lucerne. Studies for instance have shown that Lucerne exploits water deep in the soil profile and with ever dwindling water resources, it is not a suitable legume for Namibia which is the driest country in Sub Saharan Africa. Studies on plant agronomy are needed to determine ease of establishment (e.g. *O. burchellii* was more easily established from stolons with adventitious roots than seed), seed viability, adaptability low water and nutrient soils, biomass production, persistence and seed production. Other aspects that need to be investigated are drought tolerance, susceptibility to pests and diseases (for instance *R. fleckii* and *O. burchellii* pods were found to be frequently damaged by insects) and compatibility with other grass and legume species. Better understanding of these aspects will permit design of more appropriate management strategies (O' Keeffe et al., 2015) (e.g. under grazing or cut-and-carry systems) and may provide new opportunities (e.g. seed production, fodder production) and lead to more productive and sustainable use of rangelands.

## 6.7 Conclusions

1. Chemical composition of native forage legumes. With the exception of *M. candicans*, all the native forage legumes had CP > 14% which is adequate to support proper rumen functioning (Holmes et al., 2007); all the forage had NDF concentration well below the 550-600 g/kg DM that would limit intake (McDonald et al., 2010) hence they potentially could be a valuable feed resource.
2. Relative palatability. The relative palatability of *O. burchellii* and *V. lobatifolia* was similar to Lucerne; *C. argyraea* and *C. podocarpa* were consumed in proportionately small amounts in the first 5 days compared to Lucerne, but their intake increased thereafter, but was still lower ( $P < 0.05$ ) than the forage legumes *O. burchellii*, *V. lobatifolia* and Lucerne.
3. Effect of supplementation on VFI. This study demonstrated an increased intake with *L. purpureus*, *O. burchellii* and *V. lobatifolia* that was comparable to that under lucerne supplementation at 18% legume inclusion rate. At the 31% legume inclusion rate, intake was greater ( $P < 0.05$ ) under *V. lobatifolia* and *L. purpureus* compared to lucerne. The presence of saponins in lucerne may have negatively affected intake at the 31% inclusion level, which was not the case with *V. lobatifolia* or *L. purpureus*. *V. lobatifolia* and *O. burchellii* show promise for use as potential protein supplements, but need to be assayed for antinutrients.
4. Rumen fill. There may be differences in degradation rate ( $kd$ ) and extent of digestion (ratio of pdNDF to iNDF) among the forage legumes (and hence rumen fill) which influence VFI. Specifically, it is hypothesized that *V. lobatifolia* and *O. burchellii* may have lower RF effects than *L. purpureus* subsp. *purpureus*.

5. Supplementation at 18% versus 31%. Supplementation with native legumes appeared to be more beneficial on body weights of weaned Boer goats at 18% than at 31% level; optimal inclusion levels should also take into account sources of fermentable energy, amount of RUDP, intrinsic legume differences and physiological state (fast growing, pregnant or lactating) of the animal.
6. Inadequate fermentable energy. Forage legume utilization as pastures, cut forages or fodder banks can enhance animal performance, decrease expenditure on feed costs and increase profitability. Enhanced performance, however, requires ruminant animals to be supplied with adequate levels of basal feed together with supply of sources of fermentable energy and RUDP. Inadequate supply of fermentable energy may have constrained increased animal performance from the enhanced VFI that was realized with supplementation with native forage legumes; future studies should be designed to address this gap.
7. Lablab as an alternative to Lucerne in Namibia. Although *M. sativa* is the predominant forage legume utilized in feeding livestock in Namibia, the high biomass yield (Kaholongu, 2016) coupled with the high CP, NDF and ADF within acceptable ranges and its role in boosting increased voluntary intake (when used as a supplement), suggest *L. purpureus* subsp. *purpureus* as an alternative forage that should be promoted for feeding ruminants.

## 6.8 Recommendations

1. Assay for antinutrients. Fourteen forage legumes were investigated for their nutritive value in feeding ruminants and of these the most promising based on potential biomass production, plant morphology and chemical analyses were: *C. argyraea*, *R. fleckii*, *R. totta* (Thunb.) DC. var. *totta*, *V. lobatifolia* and *O. burchellii*, *L. purpureus* subsp. *uncinatus*. They need, however, to be assayed for antinutrients as part of the screening process before use in mixed diets, because they may be detrimental to rumen microorganisms and toxic to livestock.
2. Enhancing biodiversity in rangelands. All the forage legumes investigated with the exception of *M. candicans* had acceptable levels of CP for ruminant livestock. Similarly all forage legumes had acceptable concentrations of NDF (500-550g/kg DM) and ADF. The morphology, nutrient content, biomass production, antinutrients differ among them which provides opportunity for exploitation of the biological diversity for enhancing rangeland productivity. Thus combinations of these forage legumes could be explored for compatibility, adaptability and enhanced performance in diverse agro-ecological zones and different management regimes. Additionally, a diverse choice of forage legumes on offer as under pasture, permits dilution of toxins that may be present in plants and may extend the grazing season on account of different growth habits.
3. Palatability trials. The inability to get sufficient forage legume materials coupled with limited testing facilities precluded ability to carry out palatability trials on all identified species. Propagation of the forage legumes with acceptable chemical composition should be attempted so that information can be generated on agronomic characteristics

and at the same time produce ample quantities of feed for palatability and feeding trials with goats or sheep.

4. Environmental influences on estimates from chemical analyses. Variation in chemical composition and digestibility of the forage legumes can be intrinsic, but can also be due to soil and climatic conditions and to maturity of the plants from which the samples used in the analysis were obtained (Ferreira et al., 2012 pg 21; O' Keeffe et al., 2015). It would be useful to evaluate samples grown and harvested under the same location to minimize effects due to environment and plant age. Hence agronomy trials could be expanded to cover other promising legumes not covered in the Kaholongo (2016) study.

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## 8. APPENDICES

### 8.1 APPENDIX I

#### Example of SAS code used for analysis of palatability data

/\* 30-12-2016

Program: c:\students\thamina\analysis\feed12c3.sas

Data set used:

c:\students\thamina\DM consumption palatability 26042016\_radians3.xls

palatability (%)=100\*[amount of legume consumed (DM)/amount of legume offered (DM)]

5 Legumes were provided to Boer goats of about the same age on 10 consecutive days. A goat had access to each of the 5 legumes in its own pen and amount given & refusals were recorded daily.

Steps: 1) We identify best fitting covariance structure

2) We fit the model using the identified covariance structure.

Two replicates were used for determining the dry matter consumed & we use both in the analysis.

rep =replicate.

rep is nested within day:

\*/

options ps=50 ls=78 nocenter nodate;

title "Analysis of feed dm consumed";

data res1;

input sample\_id \$ legume \$ sex pen day amount1 amountr1  
amountc1 rep dm1 amountdm2 amountdmr2 amountdmc2  
crucib \$ crucibwt weight weight2 weight3 diff dmr moisture  
dmc dmcmean dmcmean2;

cards;

CA1001	CA	1	1	1	100	100	0	1	93.52	93.52	93.52	0
	5a	16.16	2	18.16	17.99	0.17	8.5	91.5	0	0	0	
CA2001	CA	1	2	1	100	0	100	1	93.32	93.32	0	93.32
	.	.	.	.	.	.	.	.	93.32	93.42	90	
CA3001	CA	1	3	1	100	82	18	1	93.52	93.52	5.74	87.78
	CA13	8.83	2	10.83	10.69	0.14	7	93	87.78	86.97	74.78	
CA4001	CA	1	4	1	100	95.05	4.95	1	93.52	93.52	3.33	90.19
	43	23.79	2	25.79	25.72	0.07	3.5	96.5	90.19325		88.91	77.41
CA5001	CA	2	5	1	100	100	0	1	93.52	93.52	93.52	0
	41	14.64	2	16.64	16.53	0.11	5.5	94.5	0	0	0	
CA6001	CA	1	6	1	100	88.38	11.62	1	93.52	93.52	5.74	87.78
	52	21.08	2	23.08	22.95	0.13	6.5	93.5	87.7753	87.68	75.64	
CA7001	CA	2	7	1	100	78.5	21.5	1	93.52	93.52	93.52	0
	CA17	8.64	2	10.64	10.55	0.09	4.5	95.5	0	44.31	38.51	
CA8001	CA	2	8	1	100	95	5	1	93.52	93.52	5.7	87.82
	CA18	10.75	2	12.75	12.63	0.12	6	94	87.82	79.88	68.56	

VL4010	VL	1	4	10	100	0	100	1	94.37	94.37	0	94.37
.	.	.	.	.	.	.	.	.	94.37	94.38	90	
VL5010	VL	2	5	10	100	0	100	1	94.37	94.37	0	94.37
.	.	.	.	.	.	.	.	.	94.37	94.38	90	
VL6010	VL	1	6	10	100	0	100	1	94.37	94.37	0	94.37
.	.	.	.	.	.	.	.	.	94.37	94.38	90	
VL7010	VL	2	7	10	100	0	100	1	94.37	94.37	0	94.37
.	.	.	.	.	.	.	.	.	94.37	94.38	90	
VL8010	VL	2	8	10	100	0	100	1	94.37	94.37	0	94.37
.	.	.	.	.	.	.	.	.	94.37	94.38	90	

```

;

/* Data follows a non normal distribution */
data res2;
set res1;
legmpen=compress(legume||pen);/*concatenate legume & pen */

if(dmcmean2 <0)then dmcmean2='.';
proc univariate normal def=5; var dmcmean2;
proc chart; hbar dmcmean2/type=pct midpoints=0 to 100 by 2;
proc sort; by legume;
run;

/*
1) Measurements on a legume-pen combination across days
are correlated. For example, CP1-day1, CP1-day2,CP1-day,...,CP1-day10
are measurements on Crotalaria podocarpa on animal 1 over the 10 days of
experimentation and they are corelated (corelation within an animal
feeding a specific legume.
Similarly there is correlation between amounts consumed of Podocarpa
across animals. Both these correlations must be accounted for in the
analysis.
*/
data res4;
set res2;
proc mixed;
class pen legume sex day legmpen;
model dmcmean2=legume day legume*day;
repeated day/ sub=legmpen type=cs r rcorr;
run;

data res4d2;
set res2;
proc mixed;
class pen legume legmpen sex day;
model dmcmean2=legume day legume*day;
repeated day/ sub=legmpen type=toeph r rcorr;
lsmeans legume day legume*day/pdiff;

/* Comparison # 1a: Difference btn treatments LC & CA
averaged over the 10 days. */
estimate 'legume CA-LC avg. over day 1-10' legume 1 0 -1;

/* Comparison # 2a: Difference btn means for
treatments LC & CA at each day. Note: Legume sequence in above

```

```

data is 1)CA 2)CP 3) LC 4) OB 5) VL. */

estimate 'legume CA-LC at day 5' legume 1 0 -1 legume*day
0 0 0 0 1 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0
0 0 0 0 -1 0 0 0 0 0;
estimate 'legume CA-LC at day 6' legume 1 0 -1 legume*day
0 0 0 0 0 1 0 0 0 0
0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 -1 0 0 0 0;
estimate 'legume CA-LC at day 7' legume 1 0 -1 legume*day
0 0 0 0 0 0 1 0 0 0
0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 -1 0 0 0;
estimate 'legume CA-LC at day 8' legume 1 0 -1 legume*day
0 0 0 0 0 0 0 1 0 0
0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 -1 0 0;
estimate 'legume CA-LC at day 9' legume 1 0 -1 legume*day
0 0 0 0 0 0 0 0 1 0
0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 -1 0;
estimate 'legume CA-LC at day 10' legume 1 0 -1 legume*day
0 0 0 0 0 0 0 0 0 1
0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 -1;
/* Comparison # 3: Difference btn means for each
day and the average of means for subsequent days
for CA. *
estimate 'day 1-days 2-10 in legume CA' day -9
1 1 1 1 1 1 1 1 1 1
legume*day
-9 1 1 1 1 1 1 1 1 1 /divisor =9;
estimate 'day 2-days 3-10 in legume CA' day 0 -8
1 1 1 1 1 1 1 1 1 1
legume*day
0 -8 1 1 1 1 1 1 1 1 /divisor =8;
estimate 'day 8-days 9-10 in legume CA' day 0 0 0
0 0 0 0 -2 1 1
legume*day
0 0 0 0 0 0 0 -2 1 1 /divisor =2;
estimate 'day.9 in legume CA' day 0 0 0
0 0 0 0 0 -1 1
legume*day
0 0 0 0 0 0 0 0 -1 1 /divisor =1;
run;

```

## 8.2 APPENDIX II

### Example of SAS code used for analysis of voluntary feed intake (VFI) data

```
/* 20-1-2017
```

```
Program: c:\student\thamina\intake16.sas
```

```
Data used in analysis:
```

```
c:\students\thamina\data\corrected_data_feeding_trial_DM_basis 27 Feb v3.1.xls
```

-Trial was carried over 74 days (14 adjustment period + 60 days trial period) starting on 14 August 2015 and ending on 27th October 2015, with goats weighed every Tuesday morning prior to feeding.

-Goats were randomly allocated by sex to the 9 different diets (control, 18%LAB, 18% OB, 18% VL, 18% LC, 31%LB, 31% OB, 31% VL, 31% LC) which correspond to treatments

```
Note:
```

```
abasalco = average of basal consumed on a daily basis by animal in a given week;
```

```
asupppo = average of supplement consumed on a daily basis by animal in a given week.
```

```
VFI= average of feed (basal+supplement) consumed by a given animal in a given week.
```

```
*/
```

```
options ps=50 ls=78 nocenter nodate;
```

```
title "Analysis of data on Voluntary Feed Intake in Boer goats ";
```

```
data res1;
```

```
input animal dob:ddmmyy8. daw:ddmmyy8. btype $ sex $ sire $ dam $ pen trt $ day basaloff supptype basalref
```

```
basalco suppref supppo totalco vfi abasalco asupppo
```

```
vfi_dm abasalco_dm asupppo_dm vfi_mbw abasalco_mbw asupppo_mbw weekwt start_wt
```

```
wk3wt;
```

```
cards;
```

143667	11/06/2014	14/08/2015	single	F	10-0296	3262	10	T1	7	950
	1	185	765	0	0	765	756.4285714	756.4285714	0	
	725.7175714	725.7175714	0	73.45404942	73.45404942	0	1	21.2		
	21.2	20.2								
143667	11/06/2014	21/08/2015	single	F	10-0296	3262	10	T1	14	900
	1	320	580	0	0	580	615.7142857	615.7142857	0	
	590.7162857	590.7162857	0	65.47978629	65.47978629	0	2	18.8		
	21.2	20.2								
143667	11/06/2014	01/09/2015	single	F	10-0296	3262	10	T1	21	1000
	1	185	815	0	0	815	722.8571429	722.8571429	0	
	693.5091429	693.5091429	0	72.78445994	72.78445994	0	3	20.2		
	21.2	20.2								
143671	11/06/2014	20/10/2015	single	M	3384	3505	19	T1	70	800
	1	130	670	0	0	670	595	595	0	570.843 570.843 0
	59.91052878	59.91052878	0	10	20.2	23.8	21.6			



143671	11/06/2014	27/10/2015	single	M	3384	3505	19	T1	74	800
	1	155	645	0	0	645	690	690	0	661.986
	63.75990336	63.75990336	0	11	22.7	23.8	21.6			661.986
143687	14/11/2014	14/08/2015	twin	M	3497	2907	1	T1	7	950
	1	305	645	0	0	645	712.1428571	712.1428571	0	
	683.2298571	683.2298571	0	54.95693085	54.95693085	0	1			28.8
	28.8	26.4								
143687	14/11/2014	21/08/2015	twin	M	3497	2907	1	T1	14	1000
	1	155	845	0	0	845	700	700	0	671.58
	60.43089044	60.43089044	0	2	24.8	28.8	26.4			671.58
143687	14/11/2014	22/09/2015	twin	M	3497	2907	1	T1	42	800
	1	0	800	0	0	800	776.4285714	776.4285714	0	
	744.9055714	744.9055714	0	55.75794715	55.75794715	0	6			31.7
	28.8	26.4								
	622.41075									
143719	23/11/2014	22/09/2015	twin	F	10-0296	3566	11	T9	42	800
	9	45	755	0	400	1155	933.5714286	562.1428571	371.4285714	
	888.6855714	539.3198571	349.3657143	108.3052802	65.72762082					42.57765942
	6	16.6	12.6	11.8						
143719	23/11/2014	29/09/2015	twin	F	10-0296	3566	11	T9	49	840
	9	540	300	0	400	700	771.4285714	417.8571429	353.5714286	
	733.4614286	400.8921429	332.5692857	92.72783503	50.68277491					42.04506012
	7	15.8	12.6	11.8						
143719	23/11/2014	06/10/2015	twin	F	10-0296	3566	11	T9	56	840
	9	240	600	30	370	970	911.4285714	574.2857143	337.1428571	
	868.0862857	550.9697143	317.1165714	112.7665267	71.57231032					41.19421643
	8	15.2	12.6	11.8						
143719	23/11/2014	13/10/2015	twin	F	10-0296	3566	11	T9	63	800
	9	350	450	0	400	850	934.8571429	567.7142857	367.1428571	
	889.9996571	544.6650857	345.3345714	128.5166484	78.65006547					49.8665829
	9	13.2	12.6	11.8						
143719	23/11/2014	20/10/2015	twin	F	10-0296	3566	11	T9	70	800
	9	300	500	110	290	790	915.7142857	595	320.7142857	
	872.5068571	570.843	301.6638571	133.6592364	87.44738094					46.2118555
	12.2	12.6	11.8							
143719	23/11/2014	27/10/2015	twin	F	10-0296	3566	11	T9	74	800
	9	280	520	225	175	695	760	518.75	241.25	724.6085
	497.68875	226.91975	99.319859	68.21666662	31.10319238					11
	12.6	11.8								14.2

;

data res2;

set res1;

if(animal='143687')then delete; /\* Excessively lost weight (5kg) during trial \*/

week2=week-2;

age=daw-dob;

wt2=wt\*.75; /\* Weights expressed on metabolic body weight basis \*/

proc freq; tables

btype sex sire dam btype\*sex trt trt\*sex week trt\*week pen supptype/norow nocol nopercnt;

run;

```

proc sort data=res2;
by trt;
run;

data res2b;
set res1;

/* Obtain plots for the consumed amounts expressed on DM basis */
proc univariate normal def=5; var abasalco_dm;
proc chart; hbar abasalco_dm/type=pct midpoints=330 to 866 by 53.6;

proc univariate normal def=5; var asuppco_dm;
proc chart; hbar asuppco_dm/type=pct midpoints=0 to 403 by 40.3;

proc univariate normal def=5; var vfi_dm;
proc chart; hbar vfi_dm/type=pct midpoints=525 to 1265 by 74;

run;
/*Fit different covariance structures to the data
to determine the best based on goodness of fit criteria
(REML logL, AIC and SBC).*/

data res3;
set res1;
proc mixed;
  class trt week sex pen btype;
  model vfi_dm= sex trt week ;
  repeated week/ sub=pen(trt) type=cs r rcorr;
run;

/* Unstructured covariance matrix;
convergence criteria met.*/
data res4;
set res1;
proc mixed;
  class trt week sex pen btype;
  model vfi_dm= sex trt week ;
  repeated week/ sub=pen(trt) type=un r rcorr;
run;

/* First order auto-regressive structure.converged. */
data res5;
set res1;
proc mixed;
  class trt week sex pen btype;
  model vfi_dm=sex trt week ;
  random animal;
  repeated week/ sub=pen(trt) type=ar(1);
run;

/* Toeplitz (banded)variance matrix. converged. */
data res6;
set res1;
proc mixed;
  class trt week sex pen btype;

```

```

model vfi_dm=sex trt week ;
repeated week/ sub=pen(trt) type=toep r rcorr;
run;

/* Standard univariate regression model. converged. */
data res7;
set res1;
proc mixed;
class trt week sex pen btype;
model vfi_dm=sex trt week ;
repeated week/ sub=pen(trt) type=simple r rcorr;
run;

/* Huynh-Feldt covariance structure
(Milliken & Johnson (1996) pg 325.
Convergence criterion met. */

data res8;
set res1;
proc mixed;
class trt week sex pen btype;
model vfi_dm=sex trt week ;
repeated week/ sub=pen(trt) type=hf r rcorr;
run;

/* Ante(1) cov structure. converged. Best covar structured based on BIC. */
data res9;
set res2;
proc mixed;
class trt week sex pen btype;
model vfi_dm=sex trt week/solution;
repeated week/ sub=pen(trt) type=ante(1) r rcorr;
lsmeans sex trt week /pdiff;

/* Compute differences btn treatments averaged over the 11 wks */
estimate 'trt control-LAB18 avg. over wks 1-11' trt 1 -1;
estimate 'trt control-OB18 avg. over wks 1-11' trt 1 0 -1;
estimate 'trt control-VL18 avg. over wks 1-11' trt 1 0 0 -1;
estimate 'trt control-LC18 avg. over wks 1-11' trt 1 0 0 0 -1;
estimate 'trt LAB18-OB18 avg. over wks 1-11' trt 0 1 -1;
estimate 'trt LAB18-VL18 avg. over wks 1-11' trt 0 1 0 -1;
estimate 'trt LAB18-LC18 avg. over wks 1-11' trt 0 1 0 0 -1;
estimate 'trt OB18-VL18 avg. over wks 1-11' trt 0 0 1 -1;
estimate 'trt OB18-LC18 avg. over wks 1-11' trt 0 0 1 0 -1;
estimate 'trt VL18-LC18 avg. over wks 1-11' trt 0 0 0 1 -1;
estimate 'trt LAB18-LC31 avg. over wks 1-11' trt 0 1 0 0 0 0 0 -1;
estimate 'trt LAB31-OB31 avg. over wks 1-11' trt 0 0 0 0 0 1 -1;
estimate 'trt LAB31-VL31 avg. over wks 1-11' trt 0 0 0 0 0 1 0 -1;
estimate 'trt LAB31-LC31 avg. over wks 1-11' trt 0 0 0 0 0 1 0 0 -1;
estimate 'trt OB31-VL31 avg. over wks 1-11' trt 0 0 0 0 0 0 1 -1;
estimate 'trt OB31-LC31 avg. over wks 1-11' trt 0 0 0 0 0 0 1 0 -1;
estimate 'trt VL31-LC31 avg. over wks 1-11' trt 0 0 0 0 0 0 0 1 -1;

estimate 'trt LB18-LB31 avg. over wks 1-11' trt 0 1 0 0 0 -1 0 0 0;
estimate 'trt OB18-OB31 avg. over wks 1-11' trt 0 0 1 0 0 0 -1 0 0;
estimate 'trt VL18-VL31 avg. over wks 1-11' trt 0 0 0 1 0 0 0 -1 0;

```

```

estimate 'trt LC18-LC31 avg. over wks 1-11' trt 0 0 0 0 1 0 0 0 -1;

estimate 'trt t1-(t2+t3+t4+t5) avg. over week 1-11' trt 4 -1 -1 -1 -1;
estimate 'trt t1-(t6+t7+t8+t9) avg. over week 1-11' trt 4 0 0 0 0 -1 -1 -1 -1;
estimate 'trt 18% vs 31% supplement avg. over week 1-11' trt 0 1 1 1 1 -1 -1 -1 -1;
estimate 'trt t1-(t2+t3+...t9) avg. over week 1-11' trt
8 -1 -1 -1 -1 -1 -1 -1 -1 ;
run;

/* ante(1). converged. Best covariance structure for
asuppc0_dm based on BIC.*/

data res11g;
set res2;
proc mixed;
class trt week sex pen btype;
model asuppc0_dm=trt week trt*week;
repeated week/ sub=pen(trt) type=ante(1) r rcorr;
lsmeans trt week trt*week;

/* Compute differences btn treatments averaged over the 11 wks */
estimate 'trt LAB18-OB18 avg. over wks 1-11' trt 0 1 -1;
estimate 'trt LAB18-VL18 avg. over wks 1-11' trt 0 1 0 -1;
estimate 'trt LAB18-LC18 avg. over wks 1-11' trt 0 1 0 0 -1;
estimate 'trt OB18-VL18 avg. over wks 1-11' trt 0 0 1 -1;
estimate 'trt OB18-LC18 avg. over wks 1-11' trt 0 0 1 0 -1;
estimate 'trt VL18-LC18 avg. over wks 1-11' trt 0 0 0 1 -1;
estimate 'trt LAB18-LC31 avg. over wks 1-11' trt 0 1 0 0 0 0 0 0 -1;
estimate 'trt LAB31-OB31 avg. over wks 1-11' trt 0 0 0 0 0 1 -1;
estimate 'trt LAB31-VL31 avg. over wks 1-11' trt 0 0 0 0 0 1 0 -1;
estimate 'trt LAB31-LC31 avg. over wks 1-11' trt 0 0 0 0 0 1 0 0 -1;
estimate 'trt OB31-VL31 avg. over wks 1-11' trt 0 0 0 0 0 0 1 -1;
estimate 'trt OB31-LC31 avg. over wks 1-11' trt 0 0 0 0 0 0 1 0 -1;
estimate 'trt VL31-LC31 avg. over wks 1-11' trt 0 0 0 0 0 0 0 1 -1;
estimate 'trt LAB18-LB31 avg. over wks 1-11' trt 0 1 0 0 0 -1 0 0 0;
estimate 'trt OB18-OB31 avg. over wks 1-11' trt 0 0 1 0 0 0 -1 0 0;
estimate 'trt LAB18-LB31 avg. over wks 1-11' trt 0 1 0 0 0 -1 0 0 0;
estimate 'trt VL18-VL31 avg. over wks 1-11' trt 0 0 0 1 0 0 0 -1 0;
estimate 'trt LC18-LC31 avg. over wks 1-11' trt 0 0 0 0 1 0 0 0 -1;
estimate 'trt 18% vs 31% supplement avg. over week 1-11' trt 0 1 1 1 1 -1 -1 -1 -1;

/* Comparison # 2a: Difference btn means for
treatments OB18 & LC18 at each week. Note: Legume sequence in above
data is 1)Control 2) LP18 3)OB18 4)VL18 5) LC18 6)LP31 7)OB31 8) VL31 9)LC31. */

estimate 'legume OB18-LC18 at week 1' trt 0 0 1 0 -1 trt*week
0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0
1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0
-1 0 0 0 0 0 0 0 0 0;
estimate 'legume OB18-LC18 at week 2' trt 0 0 1 0 -1 trt*week
0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0
0 1 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0

```

```

0 -1 0 0 0 0 0 0 0 0 0;
estimate 'legume OB18-LC18 at week 11' trt 0 0 1 0 -1 trt*week
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0

0 0 0 0 0 0 0 0 0 0 1

0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 -1;
estimate 'legume OB18-OB31 at week 1' trt 0 0 1 0 0 0 -1 0 0 trt*week
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
1 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
-1 0 0 0 0 0 0 0 0 0 0;
estimate 'legume OB18-OB31 at week 2' trt 0 0 1 0 0 0 -1 0 0 trt*week
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
0 1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
0 -1 0 0 0 0 0 0 0 0 0;
estimate 'legume OB18-OB31 at week 11' trt 0 0 1 0 0 0 -1 0 0 trt*week
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 1
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 -1;
run;

/* Get estimated effects for weights of Boer goats */
data res48c;
set res2;
if(week2 >0);
proc mixed;
class trt sire week week2 sex pen btype;
model wt=trt week2 sire btype/solution;
repeated week2 / sub=pen(trt) type=un r rcorr rc;
lsmeans trt week2 sire btype/pdiff;
estimate 'trt control-LAB18 avg. over wks 3-11' trt 1 -1;
estimate 'trt control-OB18 avg. over wks 3-11' trt 1 0 -1;
estimate 'trt control-VL18 avg. over wks 3-11' trt 1 0 0 -1;
estimate 'trt control-LC18 avg. over wks 3-11' trt 1 0 0 0 -1;
estimate 'trt control-VL31 avg. over wks 3-11' trt 1 0 0 0 0 0 -1 0;

estimate 'trt LAB18-OB18 avg. over wks 3-11' trt 0 1 -1;
estimate 'trt LAB18-VL18 avg. over wks 3-11' trt 0 1 0 -1;
estimate 'trt LAB18-LC18 avg. over wks 3-11' trt 0 1 0 0 -1;
estimate 'trt OB18-VL18 avg. over wks 3-11' trt 0 0 1 -1;
estimate 'trt OB18-LC18 avg. over wks 3-11' trt 0 0 1 0 -1;
estimate 'trt VL18-LC18 avg. over wks 3-11' trt 0 0 0 1 -1;

```

```

estimate 'trt LAB31-OB31 avg. over wks 3-11' trt 0 0 0 0 0 1 -1;
estimate 'trt LAB31-VL31 avg. over wks 3-11' trt 0 0 0 0 0 1 0 -1;
estimate 'trt LAB31-LC31 avg. over wks 3-11' trt 0 0 0 0 0 1 0 0 -1;
estimate 'trt OB31-VL31 avg. over wks 3-11' trt 0 0 0 0 0 0 1 -1;
estimate 'trt OB31-LC31 avg. over wks 3-11' trt 0 0 0 0 0 0 1 0 -1;
estimate 'trt VL31-LC31 avg. over wks 3-11' trt 0 0 0 0 0 0 0 1 -1;
estimate 'trt LAB18-LB31 avg. over wks 3-11' trt 0 1 0 0 0 -1 0 0 0;
estimate 'trt OB18-OB31 avg. over wks 3-11' trt 0 0 1 0 0 0 -1 0 0;
estimate 'trt LAB18-LB31 avg. over wks 3-11' trt 0 1 0 0 0 -1 0 0 0;
estimate 'trt VL18-VL31 avg. over wks 3-11' trt 0 0 0 1 0 0 0 -1 0;
estimate 'trt LC18-LC31 avg. over wks 3-11' trt 0 0 0 0 1 0 0 0 -1;

estimate 'trt t1-(t2+t3+t4+t5) avg. over week 3-11' trt 4 -1 -1 -1 -1;
estimate 'trt t1-(t6+t7+t8+t9) avg. over week 3-11' trt 4 0 0 0 0 -1 -1 -1 -1;
estimate 'trt 18% vs 31% supplement avg. over week 3-11' trt 0 1 1 1 1 -1 -1 -1 -1;
estimate 'trt t1-(t2+t3+...t9) avg. over week 3-11' trt
8 -1 -1 -1 -1 -1 -1 -1 -1;
run;

```

8.3 Appendix III: Pictures of some indigenous forage legumes found in Namibian



*Vigna lobatifolia* at early stage of growth



*Vigna lobatifolia* at post-flowering stage



*Otoptera burchellii* at flowering stage



*Otoptera burchellii* with pods



*Crotalaria argyrea*



*Crotalaria argyrea* with pods



*Crotalaria podocarpa*



*Crotalaria podocarpa* with pods and few flowers



Young *Rhynchosia totta* var. *totta*



*Rhynchosia totta* at flowering and pod development stages



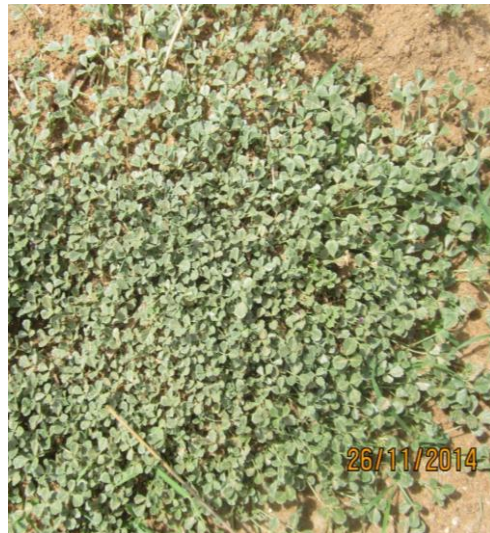
*Rhynchosia holoserica* at flowering stage



*Rhynchosia holoserica* with pods



Wild Lablab (*L. purpureus* subsp. *Uncinatus*) with pods



*Medicago laciniata*





*Meulobium candicans*



*Cullen tomentosum* at flowering stage



*Ptychlobium biflora* at early stage of growth