Nutritional characterization of browse plants harvested at different browsing heights in Eastern Cape province

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DECLARATION

I, Thabiso M. Sebolai, declare that this PhD thesis is my own original and independent research work. The thesis was carried out under the supervision of Profs. V. Mlambo, S. Beyene and R. Madibela. This thesis or any part of it has not been previously submitted by me for any degree or examination to another faculty or University. The research work reported in this thesis does not contain any person's data, pictures, graphs or other information unless specifically acknowledged as being sourced from those persons.

Signed:_____ Date: 27/04/2018

Thabiso M. Sebolai (candidate)

As the candidate's supervisor I agree, on behalf of all the members of the supervisory team, to the submission of this thesis.

Signed:_____ Date: 27/04/2018

Prof. V. Mlambo (Promoter)

DEDICATION

This work is dedicated to my mother, Phomolo Sebolai, who provided me with love and care and ensured that I reach this point of my life. The Sebolai, Pelekekae, Masoloko and Joseph families who played crucial role on my upbringing especially the late Mrs Selwana Emelda Sebolai and Mrs Ada Adelade Pelekekae, who supported and inspired me throughout all my education. All this is for you.

GENERAL ABSTRACT

The main objective of this study was to determine the influence of browse plant species and harvesting heights [browsable (<1.5 m) and non-browsable (>1.5 m)] on the nutritional value of browse plant leaves. Leaves samples were collected from *Maytenus capitata, Olea africana, Coddia rudis, Carissa macrocarpa, Rhus refracta, Ziziphus mucronata, Boscia oliedes, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida.* Chapter 1 of this thesis comprises of background information, defines the statement of the problem and the justification of the study. Chapter 2 is the review of related literature on the importance of browse plant leaves, factors affecting chemical composition and the response of plant leaves to such factors. In Chapter 3, samples were analysed for proximate components, soluble phenolics, condensed tannins and minerals. The results showed that plant species had an effect ($P < 0.05$) on fibre and mineral content of browse leaves. Plant species and interaction between plant species and harvesting height had a significant ($P < 0.05$) influence in terms of total N, total phenolic content, while harvesting height did not influence ($P > 0.05$) these substances. The leaves contained moderate to high levels of N which ranged from 81.5 g/kg N (*Carissa macrocarpa*) to 168.6 g/kg N (*Z. mucronata*) at both heights enough to meet level required by microbes in the rumen, making them potential sources of supplementary protein during the dry seasons. Total phenol results show the interaction of tree species and harvesting height. Tree species and harvesting height affected ($P<0.05$) condensed tannin (CTs) content as on average the leaves of non-browsable height (0.61 $AU_{550\ nm/200\ mg}$) was higher than that of leaves at browsable height (0.55 $AU_{550\ nm/200\ mg}$). Chapter 4 investigated the effects of plant species and harvesting height on buffer nitrogen solubility, dry matter and nitrogen degradability of plant leaves. The results revealed that the browse plants and interaction between browse species × harvesting height had a significant impact on nitrogen solubility index of browse species. The study also showed that plant species

and harvesting height and their interaction had influence ($P < 0.05$) on IVDMD and IVND at 12, 24 and 36 hours of incubation. At non-browsable height, *B. oleoides* (291.2 g/kg DM) had the highest ($P < 0.05$) immediately degradable dry matter 'a' fractions while at browsable height *R. refracta* (292.4 g/kg DM) had the highest fraction 'a'. Leaves of *E. rigida* harvested from both non-browsable (455.8 g/kg DM) and browsable (729.5 g/kg DM) height had the highest degradable part of insoluble 'b' fraction of dry matter. The IVDMD of browse leaves was low for both plant heights, which could be due to high fibre and moderate to high levels of tannins in the leaves. The results obtained in Chapter 3 and 4 were used as reference values to calibrate and validate the NIRS as a possible tool to predict the nutritive value of browse plant leaves. Leaves were scanned (32 scans per spectra), and spectra recorded at intervals of 2 nm using the SpectraStar XL then spectral data were recorded in diffuse reflectance and expressed as log (1/R). The next step was to transfer the reference values into the NIRS spectral data where they were used to generate calibration models with the aid of UCal software. Calibration models were validated using reference values and respective spectral data from an independent set of browse leaves from different areas. All chemical parameters had good calibration statistics with high R^2 values (>0.8) and low standard error of calibration (41.58). External validation revealed that the prediction accuracy of calibration model for total N level was high since it was able to explain 88% of the variation in this parameter in independent samples and had a small standard error of prediction (SEP) of 16.34. However, validation statistics were poor for fibre, which could be due to errors in the determination of fibre fractions during laboratory analysis. The results also show that only the OM and N calibration models generated from this study can be utilized to accurately predict these components in browse plant leaves. Thus it is concluded that NIRS can be used to rapidly predict total N and OM content of these substrates that are frequently used as protein supplements in ruminants and other herbivores.

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LIST OF ABBREVIATIONS

OM	Organic Matter
NDF	Neutral Detergent Fibre
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
CP	Crude Protein
SPh	Soluble Phenolics
TAE	Tannic Acid Equivalents
CT	Condensed Tannins
HT	Hydrolysable Tannins
SCT	Soluble Condensed Tannins
SECV	Standard Error of Cross Validation
NSI	Nitrogen Solubility Index
INDMD	*In vitro* ruminal dry matter degradability
IVND	*In vitro* ruminal Nitrogen Degradability
NIRS	Near Infrared Spectroscopy

1. CHAPTER 1: INTRODUCTION

1.1 Background

Feed resource shortage is one of the major challenges that smallholder livestock farmers face, especially those whose animals depend solely on communal rangelands. Rangelands play a crucial role in the socio-economy of many countries by supplying about 70% of feed needed by domestic ruminants (Tolera *et al.,* 1997). In Botswana, browse leaves and pods provides over 60 per cent of the forage requirement of the diet of goats (Aganga & Tshwenyane, 2003). The nutritive value of leaves of browse plants is generally higher than that of grasses (monocots) and they are able to retain their nutritional status for the whole dry season when grasses dry up and decline both in quality and availability (Boufennara *et al.,* 2012). It is important for farmers to have some knowledge and understanding of nutritional value of their grazing areas as these will assist in proper feeding and supplementation if necessary.

Chemical composition of forage varies as the plant grows. This could be due to the fact that the plant is exposed to different biotic and abiotic factors during growth. Kraus *et al.* (2003) reported that nutritional composition of forage is greatly affected by biotic factors such as stage of growth, herbivory and abiotic factors, which include altitude, soil characteristics (fertility, moisture, pH and structure), temperature, carbon dioxide and ozone. Plants respond in different ways to environmental stressors and one of the ways is biosynthesis of plant secondary metabolites such as phenolics, terpenes and alkaloids (Rispail *et al.*, 2005; Bourgaud *et al.*, 2001). Secondary plant metabolites are present in specialized cells that are not directly essential for basic photosynthetic or respiratory metabolism but are thought to be required for plant's survival in the environment (Lattanzio, 2013). They are structurally and chemically much more diverse than the primary metabolites. It is crucial to understand that

plants are genetically diverse and their responses to such stressors differ as well. Research has demonstrated that plants have two main mechanisms of response to stress which are inductive and constitutive (Frost *et al.*, 2008). Inductive mechanism is whereby the plant is induced by stressing factors to synthesize chemical substances or physically as a defending strategy while constitutive is always present (Frost *et al.,* 2008). Plants with inductive defense mechanisms may suffer some damage since the defense is not always present, the attack or stress is the one that stimulates activation of the defense, although it compensate for that by priming.

Phenolics are the most abundant secondary plant metabolites and can be classified as non-soluble (e.g. tannins and lignin) and soluble (e.g. flavonoids) (Rispail *et al.*, 2005). Phenolic compounds are the secondary metabolites of plant which help in their normal growth and development (Kondakova *et al.*, 2009). Cartea *et al.* (2011) explained that phenolic compounds‖ is a generic term that denotes a large number of compounds (more than 8,000) widely spread throughout the plant kingdom and their distinctive trait is at least one aromatic ring with one or more attached hydroxyl groups. They range from simple, low molecular-weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols. Furthermore, amongst the phenolics, tannins are the most abundant secondary metabolites synthesized by plants, commonly ranging from 5 to 10% dry weight of tree leaves. Plant secondary metabolites such as phenols and tannins are biosynthesized as a response of plants to various stresses. These secondary metabolites are known to have both negative and positive effects on herbivore nutrition. They have been found to control parasitic load in animals, reduce methane production and occurrence of bloat in ruminants feeding on lush legumes as well as increase rumen bypass protein (Patra & Saxena, 2011).

1.2 Problem Statement

In the Eastern Cape, there is little information on the nutritional characteristics of browse plants of various species, which would aid in their utilization as sources of nutrients for ruminant and non-ruminant herbivores. This leads to inefficient utilization of browse leaves, which subsequently negatively affect livestock performance. The livestock industry is faced with the challenge of a shortage of both quantity and quality feeds which is escalated by seasonality and climate change. This necessitates full understanding of nutritional value of available feed resources that farmers have at their disposal since there are wide variations between chemical components of browse species (Apori *et al.*, 1998). The understanding of how plant secondary compounds are distributed in browse plants is little understood. As plants respond to stress factors it raises questions on whether at different browse heights the content of plant secondary compounds in available browse material are similar. This is important to understand as it has an important implication on the quality of browse taken by the animal during feeding especially where there are animals in a herd of different browse heights.

The conventional feed analysis methods which have been used for analysis of livestock feeds have been so crucial on feed analysis thus enhancing knowledge about feed chemistry but they have their challenges. Limitations of conventional methods ranges from time consuming, expensive, require skilled personnel for mixing reagents and operation of instruments and production of pollutants. These necessitated invention of technology that is efficient (cost effective and fast), require less skill to analyse samples and environmentally friendly, hence invention of near infrared spectroscopy (NIRS).

1.3 Justification

Livestock feeding is easily the biggest cost in farm animal production. It constitutes about 70% of the rearing costs, so reducing the cost of feeding increases profitability. One of the ways of cutting feeding costs is the use of locally available, inexpensive and non-conventional feed resources such as browse plants. However, the nutritional composition of these browse plants differs widely as they are exposed to various biotic and abiotic conditions and their responses differs due to their genetic variation. This necessitates nutritional assessment and characterization of various plant parts such as leaves which are considered nutritious as mostly livestock feed on them. Nutritional assessment of these browse plant leaves at different browsing heights will allow informed utilization of these non-conventional feed. Browse plants play a crucial role as the main source of nutrients to ruminants reared under arid and semi-arid environments, where inadequate feeds are a major constraint to livestock production (Aganga & Tshwenyane, 2003). They retain higher protein and mineral levels during growth than do grasses which deteriorate in quality rapidly with progress to maturity (Shelton, 2004).

Knowledge and understanding of nutritional value of feed is crucial on efficient utilization of the feeds, hence the need for farmers to acquire such knowledge. This knowledge can only be available when feeds are analysed, but due to high cost of feed analysis farmers can barely afford such procedures. Hence the need for use of Near infrared spectroscopy which is efficient (rapid and affordable), non destructive and require less skill to obtain results (De Boever *et al.*, 1995, Swart *et al.,* 2012; Mnisi, 2015).

1.4 Objectives

1. To nutritionally characterise (chemical composition, nitrogen buffer solubility, *in vitro* ruminal dry matter and nitrogen degradability) of *Maytenus capitata, Olea africana, Coddia rudis, Carissa, Rhus refracta, Ziziphus mucronata, Boscia oleoides, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida* browse leaves harvested from two browsing heights in the Eastern Cape province, Republic of South Africa.

2. To calibrate and validate the NIRS technique for use in predicting nutritional parameters of *Maytenus capitata, Olea africana, Coddia rudis, Carissa, Rhus refracta, Ziziphus mucronata, Boscia oleoides, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida* browse leaves sampled from two browsing heights in the Eastern Cape province.

1.5 Research questions

The study was designed to answer the following research questions:

1. Are there any species variations in terms of nutritional composition (chemical components, protein solubility, *in vitro* ruminal dry matter and nitrogen degradability) of browse leaves harvested at different browsing heights?
2. Does the NIRS method provide spectral variables with nonzero coefficients, which may be used to accurately estimate the nutritive value of browse plant leaves?

2. CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Browse plants are some of the major sources of nutritious feed for ruminant animals. Although they are essential in animal production, not much work has been done to nutritionally characterize a majority of the available species that are used by domesticated and wild animals. The leaves of these plants have been acknowledged for high crude protein and phenolics, especially tannins. The protein content of most browse leaves is above the level required by ruminal microbes, but it has been reported that they form complexes with tannins which reduce the protein degradation in the rumen, but is mostly hydrolyzed in the small intestine. Hydrolyzation of tannin-protein complexes in the intestines benefits the animal by direct acquisition of dietary amino acids from plant. This is essential for high producing ruminants and released tannins are toxic to parasitic worms (Min & Hart, 2002). However, the tannin-protein complex disadvantages livestock feeding on fibrous diets with low levels of protein thereby not meeting ruminal microbial requirement resulting in reduced fibre digestibility. The concentration of plant photosynthates is affected by various factors such as the plant growth and leaf age which alters their level in plants, hence the need to determine them at different height.

2.2 Common browse plants of the Eastern Cape Province

2.2.1 Phyllanthus verrucosus

Phyllanthus verrucosus is a shrub growing to a height ranging from 1.22–2.4 m high. It belongs to the family Euphorbiaceae. It has many straight branches, covered with a grey bark, verrucose with conspicuous lenticels; lateral twigs increasing in length towards the base of each main shoot. Branchlets that are flowering are very short, each with a cluster of 3–5 leaves. The leaves of this tree are obovate or obovate-elliptic, rounded and frequently slightly retuse at the apex,

obtuse or subcuneate at the base, 5–15.2 cm long (Royal Botanic Gardens, 2006).

2.2.2 Ehretia rigida

Ehretia is a pantropical genus with about 50 species (Miller, 1989). *Ehretia rigida* (Puzzle bush) is one of the 50 genus, it is a small, multi-stemmed deciduous tree with a tangled growth habit. It belongs to the Boraginaceae or borage family. Ndou (2003) elaborated that the genera of this tree (Ehretia) were named after botanical artist, R.D Ehret in 18th century while species (rigida) refers to stiffness, describing the hard leaves. *Ehretia rigida* is normally used for traditional, medicinal purpose. It is also very attractive to most birds and insects. The puzzle bush is hardy and drought tolerant and grows easily. *Ehretia rigida* serves as a feed resource to both domesticated and wild animals, such as the kudu, nyala, bushbuck, impala and grey duiker.

2.2.3 Coddia rudis

Coddia rudis belongs to Rubiaceae (Coffee family) and its common name is Small bone apple. Sepheka (2012) explained that *C. rudis* is a short, dense multi-stemmed fast growing shrub, normally up to 3 m in height. The main stem of *C. rudis* is normally short, with arching branches. It grows stiffly upwards, outwards and finally downwards and it forms a compact shrub if browsed. The leaves are opposite or borne in dense clusters on dwarf side twigs. The leaves are simple, broadly obovate, usually 20 mm wide x 15 mm long, shiny dark green above, paler below. *Coddia rudis* is distributed from the Eastern Cape through KwaZulu-Natal to the eastern Lowveld and Swaziland, on forest margins in bush clumps beneath tall acacias and among rocks (Sepheka, 2012). It can grow well in sandy soil, strong wind, limited rainfall and intense sunlight. It can tolerate long periods of drought and high temperatures without extra water. The leaves are heavily browsed by game, and it is one of the five browse species that van

Lieverloo *et al.* (2009) reported that they dominated the diet of black rhino and makes up to about 68.8% of total consumed biomass.

2.2.4 Boscia oleoides

Boscia oleoides is a dicotyledonous tree belonging to Brassicaceae family and it's commonly called Bastard Shepherd Tree (Eng), Karoo Shepherd Tree (Eng), Karoo-witgat (Afrk). Both the Latin and the Afrikaans (Witgat) names refer to its conspicuous white trunk, hence its Latin species (albi'- white, trunca'– trunk). Its English name describes this evergreen tree that stands out as a green well-shaped canopy that often provides the only shade in arid environments (Samara, 2015). This tree grows well on well-drained, sandy or rocky soils and is widespread in dry, open woodland and bushveld. It is most common in rocky and lime-bank terrains. It is of small to medium height (2-8 m), but basically grows higher than 4 meters in the Karoo. Its leaves are rich in protein, nutritious and palatable and it always shows a clear umbrella-like browse- line.

2.2.5 Rhus refracta

Rhus refracta belong to family Anacardiaceae, and its common name is Toxicodendron refractum. Roux (2003) indicated that *R. refracta* is a multi-branched squarrose short shrub that can grow up to 3 m. Its leaves are trifoliate, petiolate petiole semi-terete and shallowly canaliculated. It is widespread in the Eastern Cape province between the Sundays and Kei River from the coast to near Cradock inland. Its range is extended north-eastwards by a few collections in Transkei and south-westwards by isolated collections near Willowmore and Plettenberg Bay in the southern Cape.

2.2.6 *Grewia robusta*

Grewia robusta is a short multi-stemmed shrub growing up to 3 m high. Its leaves often clustered on abbreviated side-shoots, broadly elliptic to ovate or almost round, 13–25 x 10–20 mm. The petiole (leaf stalk) is very short, bears round and fleshy drupe fruits, which are reddish brown. *Grewia robusta* is one of the five browse species that van Lieverloo *et al.* (2009) reported to have dominated the diet of black rhino which together comprised 68.8% in terms of total ingested biomass. They indicated that preference for *Grewia spp.* has also been reported in other studies (van Lieverloo *et al.,* 2009).

2.2.7 *Carissa macrocapra*

Carissa macrocapra tree species belong to Apocyanaceae family which consists of 200 genera and 250 species. It is a spiny evergreen or a scrambling growing bush of 5 m height. Its barks are grey, smooth, with straight woody spines, sometimes forked up to 5 cm long. Leaves are glossy green, base rounded and apex pointed. Research has been done on different parts including the bark, roots and fruit of this plant but less on leaves. Al-Youssef & Hassan (2014) reported that *Carissa* is a genus that is a rich source of various natural classes of compounds such as phenolic compounds, flavonoids and lignans.

2.2.8 *Olea africana*

Olea africana is a subspecies of *Olea europaea* and belonging to the Oleaceae family. Joffe (2002) indicated that there are four species of *Olea* in South Africa and *O. africana* is one of them. Its common name is Wild olive, it is a neatly shaped, evergreen tree with a dense spreading crown (9 x 12 m) of glossy grey-green to dark-green foliage and it grows up to 15 m height. It adapts well in a variety of habitats, as it is a drought tolerant and wind resistant browse tree. *Olea*

africana is often found near water, on rocky hillsides, on stream banks. Leaves are grey-green to dark-green above and greyish below. Leaves are browsed by game and livestock and play a crucial role in very dry areas during dry seasons because it is extremely hardy and is an excellent fodder tree (Joffe, 2002).

2.2.9 Maytenus capitata

Maytenus capitata belongs to Celastraceae family (Ecklon, 2012). Maddock *et al.* (1995) reported that *M. capitata* and *G. robusta* were amongst 12 plant species fed to captive black rhinoceros (*Diceros bicornis). Maytenus capitata* was one of the four most consumed species, while *G. robusta* was eaten readily but less than *M. capitata.*

2.2.10 Ziziphus mucronata

This browse species is normally a shrub or medium-sized tree growing up to nine metres tall with an irregular crown, dense spreading and drooping branches (Heuze & Tran, 2015) .Its common name is buffalo thorn (Cape thorn) and it belongs to the Rhamnaceae family (Orwa *et al.,* 2009). The trunk of *Z. mucronata* is short, with diameter of up to 40 cm and frequently crooked, with branches spreading, often drooping, branching well above ground with grey brown bark. Its leaves are ovate to broadly ovate, mucronate, 2.5-8 x 1.9-8 cm, shiny, densely hairy to quite smooth (Orwa *et al.,* 2009). Aganga & Mosase (2001) indicated that this tree is valued as foliage for browsers as the young leaves are not very palatable but are nutritious.

2.3 Types of ruminants and their morpho-physiological variations

Ruminants are divided into grazers, browsers (concentrate selectors), and intemediate (opportunistic) feeders according to their feeding habits (Münnich, 2008). Hofmann (1987) used

the eco-physiological adaptation concept to explain the diversity in ruminant dietary patterns, classifying them in three overlapping morpho-physiological feeding types, although they have been criticized by other researchers (Lamy *et al.,* 2012). Lashley *et al.* (2014) indicated that concentrate selectors account for more than 40% of ruminants worldwide, and these types of ruminants are equipped with a digestive tract that is well adapted to process highly digestible forages rich in soluble cell contents, low in cell wall contents. The rate of fermentation is rapid while rumination is less important, feeding on smaller amounts frequently.

The other 60% of ruminants are intermediate browsers (35%) and roughage, grass eaters (25%), their digestive system are well adapted to digest lignified material, requiring a less selective diet but greater intake rates and longer retention times to extract nutrients (Hofmann, 1987; Lashley *et al.,* 2014). Sanon (2007) indicated that the feeding behaviour of natural browsers such as goats have anatomical and physiological features that sustain their feeding strategy. They have small mouth, with flexible and mobile upper lips with tongues which enable this class of ruminants to nibble and pick small leaves between thorns, flowers, fruits and other most nutritious plant parts. Lamy *et al.* (2012) emphasized that the oral cavity plays a crucial role in the identification, recognition and decision processes during feed selection, whether to ingest or reject.

Intermediate feeders such as goats have selective behaviour. Sun *et al.* (2014) reported that goats selected plant parts that had higher crude protein (CP) and lower acid detergent fiber (ADF) and neutral detergent fiber (NDF) than the whole plant, especially in the autumn and winter. Min & Hart (2003) indicated that some ruminants, especially concentrate selectors and intemediate feeders are able to tolerate and browse high tannin forage as they produce more protein-rich saliva. They have well developed parotid glands, responsible for the production and secretion of salivary

proteins (proline) with a high affinity for tannins. A tannin-binding salivary protein (TBSP) is considered as a defense against the potential toxic and/or antinutritive substances. Saliva has a major importance for diet adjustment as it serves as a physiological buffer (pH 8.2) against variations between the animal's external and internal milieus.

The diet of grazers is basically grass with little dietary selectivity. This class of ruminant has large reticulo-rumens, which afford them to retain fibrous forages for a longer time (Hofmann, 1987). This allows degradation of diets that have high percentage of cell wall contents, including cellulolytic and amylolytic activity and relatively lignified forages.

2.4 Chemical composition of browse plants

Browse plants play a pivotal role in ruminant nutrition in most parts of the world, especially during dry conditions as they are the main source of quality feed especially for small-scale farmers who depend solely on rangelands (Tolera, 1997). Their leaves are able to remain green and maintain high nutritional value over a longer period during the dry season, while most of the grass pasture deteriorates in quality rapidly during such periods. Most browse plants contain moderate to high crude protein that is above the minimum of 80 g/kg DM required to meet ruminal microbes (Leng, 1990). Sanon (2007) also noted that high CP level in browse plants is well documented and is one of the main distinct characteristics of browse compared to mature grasses. These indicate that browse plants serve a crucial part of supporting and supplementing livestock during the dry season. Although the nutritive value of conventional feeds for animals has been studied extensively, much less information is available about the nutritive value of alternative feeds such as forage browse in other areas of the country (Ammar *et al.*, 2004), particularly browse plants which are not utilized as human foods thus not in direct competition

with people (Dupe *et al.,* 2015). Many wild browse and bush species are undervalued because of insufficient knowledge about their potential feeding value (Boufennara *et al.,* 2012). Most of browse plants contain higher level of CP than grass, and they contain considerable levels of condensed tannins which play crucial role in both animal health and nutrition. Browse plants contain considerable condensed tannins which have been reported to have some benefits in ruminant nutrition. Patra & Saxena (2011) reported a reduced worm load in livestock supplemented with browse plants.

The challenge of availability of quality livestock feed is aggravated in arid, semi-arid and tropical regions susceptible to scarce and erratic rainfall which limits the growth of herbaceous species and biomass yield in rangelands (Boufennara *et al.,* 2012). One of the main constraints in animal production in Sub-Saharan Africa is animal nutrition, as inadequate feeding can lead to reproductive wastage, low birth weights, high infant mortality, etc. (Sumberg, 1985). Thus, livestock in such regions have to survive on recurrent shortage of feed resources of insufficient nutritional value for most parts of the year, as the nutritional value of grass declines drastically during the dry season (Robles *et al.*, 2008; Yayneshet *et al.,* 2009).

2.5 Overcoming forage seasonality

Forage trees and shrubs play crucial roles in the diet of domestic ruminants which mainly rely on rangelands. Dicko & Sikena (1991) reported that browse plants are the major sources of proteins, minerals and vitamins in ruminant diet during the dry season especially for livestock that depends exclusively on rangelands. The availability and quality of the pasture varies with seasonal or climatic changes, with poor fibrous grass during the dry season. Moya-Rodríguez *et al.* (2002) indicated that the quality of a diet for ruminants depends on the chemical composition of

forage available to them. Giridhar & Samireddypalle (2015) stated that climate change has the potential to influence and change the availability and reliability of forage production and the quality of forage. Fluctuations in distribution and intensity of rainfall during the rainy season in several regions of the world have effects on the forage production and quality. Blair *et al.* (1977) observed that browse leaves and twig tips were the most abundant, nutritious and digestible forage throughout the year. During dry periods and long droughts, the availability and quality of pasture deteriorates, even grazers like cattle start browsing. So the browse plant's leaves and to some extent pods provide quality supplementation of poor fibrous pasture during dry periods. As noted by Tolera (1997), browse plants can play an important role in providing high quality fodder for ruminants in most parts of the world during dry conditions.

2.6 Phenolics content of browse plants

Browse plants are characterized by having a considerable amount of CP and phenolics. Phenolic compounds are a large class of plant secondary metabolites, showing a diversity of structures, from rather simple structures, e.g. phenolic acids, through polyphenols such as flavonoids (Cheynier, 2012). Phenolics are the most abundant secondary metabolites in plants and can be classified as non-soluble (e.g. tannins and lignin) and soluble (e.g. flavone) (Rispail *et al.*, 2005). Hutzler *et al.* (1998) indicated that phenolic compounds are part of the interactions between plants and their biotic and abiotic environment. They accumulate in various plant tissues and cells during ontogenesis and are influenced by different environmental stimuli.

Amongst the phenolics, tannins are the most abundant secondary metabolites synthesized by plants, commonly ranging from 5 to 10% dry weight of tree leaves. Khanbabaee & van Ree (2001) explained that the name 'tannin' was derived from the French 'tanin' (tanning substance)

and is used for a range of natural polyphenols. It was given to plant extracts that exhibited astringency, without knowing their chemical structures, (Okuda & Ito, 2011). They are found in higher plants and are divided into two major types termed condensed and hydrolyzable tannins (Kraus *et al.,* 2003).

2.6.1 Tannins

Acero *et al.* (2010) explained that tannins are polyphenolic compounds that are able to react and form complexes with proteins and play a role in ecological processes such as decomposition, nutrient cycling, nitrogen sequestration and microbial activity. Tannins occur in cell vacuoles of many feeds such as fodder legumes, browse leaves and fruits (Barry & McNabb, 1999; Mueller-Harvey, 2006). The chemistry of CT is complex. First, there are differences in the hydroxylation of the B-ring of the flavan-3-ol monomer units.

2.6.1.1 Condensed Tannins

Condensed tannins which are also known as proanthocyanids, and to be bioactive ingredients in forage consumed by animals. He *et al.* (2008) explained that proanthocyanidins refer to the release of anthocyanidins from extension positions after being boiled with strong mineral acid. Correspondingly, procyanidins designate oligomers and polymers with 3‘,4‘-dihydroxyl pattern ((+)-catechin and/or (-)-epicatechin) extension units, while propelargonidins or prodelphinidins designate oligomers and polymers. Patra & Saxena (2011) and Saito *et al.* (2013) described proanthocyanidins mainly as polymers of the flavan-3-ol (epi) catechin and (epi) gallocatechin units, which are linked by C4-C8 and/or C4-C6 interflavonoid linkages.

Condensed tannins
flavan-3-ols
Proanthocyanidins

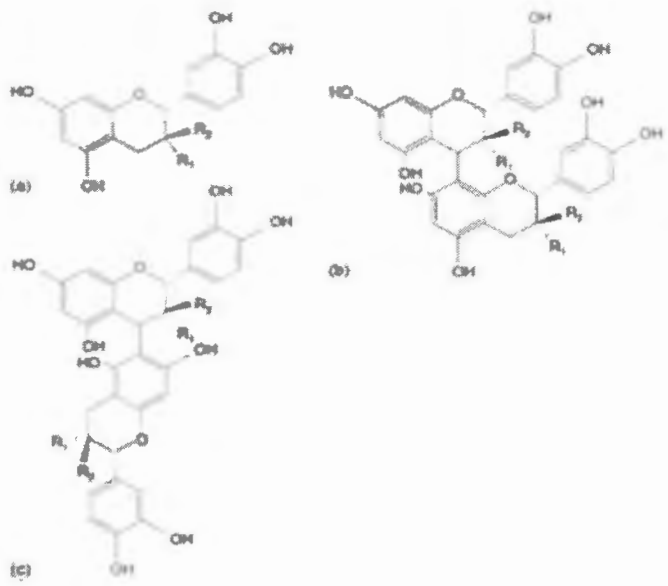

Figure 2.1: Structure of Flavan-3-ol monomers and dimers. (A) –Epicatechin with R1=OH and R2=H or (+)-catechin with R1=H and R2=OH; (B) procyanidin (4b-8)-dimer; (C) procyanidin (4b-6)-dimer. Condensed tannins chemical structure (Okuda *et al.*, 1995)

2.6.1.2 Hydrolyzable tannin

Hydrolyzable tannin molecules comprise a carbohydrate (generally D -glucose) as a central core (Min & Hart, 2003). The hydroxyl groups of these carbohydrates are esterified with phenolic groups, such as ellagic acid or gallic acid. Hydrolyzable tannins can be further metabolized to compounds such as pyrogallol (Meiser, 2000), which are potentially toxic to ruminants. Makkar (2003) also cautioned that tannins can be poisonous if consumed in large quantities. Mueller-Harvely (2001) indicated that hydrolysable tannins have not been given much attention due to complexity of analysing this type of tannins.

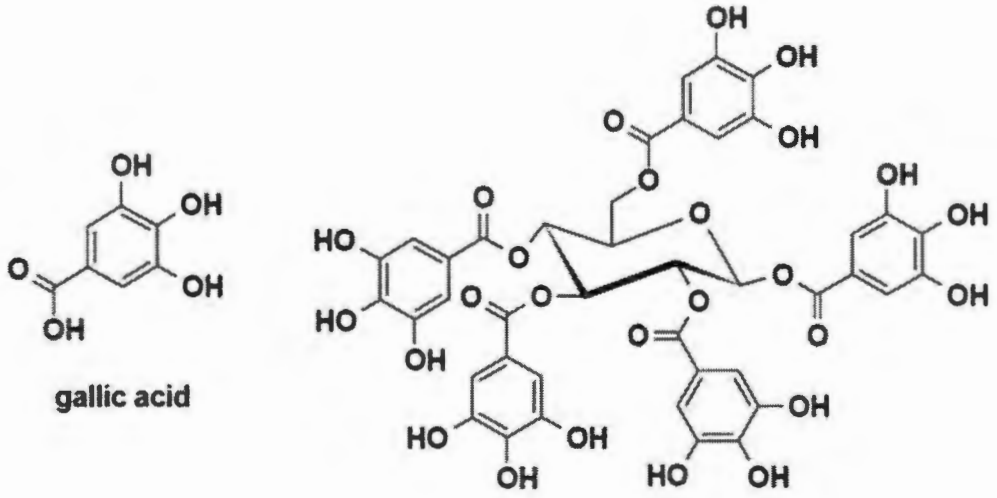

Figure 2.2: Hydrolysable tannins (Hagerman, 2010)

2.6.2 Condensed tannins in animal health and nutrition

Browse plant leaves contain condensed tannins which are nutritionally important compounds with both negative and positive effects on herbivore nutrition. Browse plants contain moderate to high protein and condensed tannins, which can bind to form tannin-proteins complexes. Tanner *et al.* (1990) indicated that the aromatic rings and multiplicity of hydroxyl groups on the flavanol subunits can interact with carbohydrates, protein amino acid residues by hydrophobic and hydrogen bonds. The properties of tannins such as molecular weight, which affect the strength of complexes formed with protein and carbohydrates lead to the positive and negative effects.

2.6.3 Nutritional importance of condensed tannins in browse plants

Browse plants are a source of protein which is one of the most crucial and expensive nutrients in livestock diets. For the efficient use of protein especially in ruminants it is important to

balance rumen degradable and undegradable protein. It has been established that condensed tannins have the ability to reduce degradation of protein in the rumen, leading to increased flow of dietary amino acids to the intestine for absorption (Waghorn *et al.,* 1994). Min *et al.* (2003) noted that the CTs bind with proteins and other molecules tightly at near-neutral pH, such as occurs in the rumen, which dissociate in the acidic pH environment of the abomasum, freeing them for digestion.

2.6.3.1 Prevention of bloat

Bloat is one of the nutritional disorders that mostly occur in grazing ruminants especially those exposed to lushy legumes (Mcdonald *et al.*, 2002). It normally occurs when ruminants consume very high soluble forage proteins, which leads to formation of a stable foam in the rumen. Tian *et al.* (2008) explained that rapid rumen degradation of rich highly digestible proteinaceous feed led to production of large volume of methane which can be trapped in the protein foam, resulting in the potentially lethal condition of pasture bloat. Bloat is very prevalent to cattle feeding on lush legumes, especially in spring (Barry & MacNabb, 1999). Since condensed tannins form complexes that reduce protein solubility in the rumen this eliminate bloat, as Anuraga *et al.* (1993) Min (2003) and Mueller-Harvey (2006) explained that tannins can render feed constituents less digestible by binding to them. Protein digestibility tends to be reduced most, but carbohydrate, starch and cell wall digestibility can also be affected. Li *et al.* (1996) indicated that the minimum plant condensed tannins level required to prevent bloat due to forage was not known, but recently 5 g CT/kg DM or greater was proposed but should be within tolerant level as high levels tend to suppress feed intake (Barry & MacNabb, 1999).

2.6.3.2 Parasitic and worm load control

Grazing ruminants are susceptible to several diseases, some of which have a nutritional component. One such condition is internal parasite infestation in grazing sheep, cattle, deer and goats (Barry & McNabb, 1999). Internal parasitic load is currently controlled by use of chemicals such as anthelmintics which are drenched while others are injectable to kill the parasites (Barry & McNabb, 1999). The use of these chemical remedies controls both conditions in the short term but have long-term problems. Repeated use of anthelmintics may lead to drug resistance by the parasites and pathogen and pose a threat of reaching end users in the food chain (Aganga *et al.*, 2006). Esterre & Jamet (1987) and Keiser *et al.* (2012) also emphasized the concerns that anthelmintics and the drugs recently introduced on the human medical market (flubendazole, albendazole, praziquantel) are largely used in veterinary medicine for many years. End users of animal by-products have also shown concern about the use of chemicals and drugs in livestock such as antibiotics, ionophores, methane inhibitors and defaunating agents for promotion of animal performance due to possibility of transfer of their residue to humans resulting in drug resistance (Barry & McNabb, 1999; Patra & Saxena, 2011). These effects have resulted in a reconsideration of control measures, in specifically the development of new control measures that are more nutritionally based and ecologically sustainable. van den Bogaard & Stobberingh (2000) noticed that most retrospective and prospective studies show that after the introduction of an antibiotic not only the level of resistance of pathogenic bacteria, but also of commensal bacteria increased. These show the need to come up with alternative options of controlling and treatment of livestock that will not have negative impact on end users of livestock product and in this case condensed tannins have shown the ability to control some of the internal parasites that are treated by drugs.

Min (2003) noticed increasing evidence that gastrointestinal parasite (GIP) control programs based on dewormers are failing because of increased worm resistance, thus, alternative GIP control strategies are necessary. Williams *et al.* (2014) concluded that condensed tannins can have potent, direct anthelmintic effects against *Ascaris suum*, as they reduced migratory ability of newly hatched third-stage larvae and reduced motility and survival of fourth-stage larvae recovered from pigs. They used transmission electron microscopy which showed that CTs significantly damaged the cuticle and digestive tissues of the larvae, but the effectiveness of the anthelmintic effect was related to the polymer size of the tannin molecule. Moreover, the identity of the monomeric structural units of tannin polymers may also have an influence as gallocatech and epigallocatechin monomers exerted significant anthelmintic activity whereas catechin and epicatechin monomers did not.

2.6.3.3 Condensed tannins and enteric methanogenesis

One of the challenges that the world is facing at the moment is the warming of the globe which is mainly caused by greenhouse gases. According to Inthapanya *et al.* (2011) agricultural practices accounts for 10-12% of total global anthropogenic greenhouse gas emissions, of which 50% is methane (CH_4). Ruminants are a major source of anthropogenic CH_4, contributing about 33% annually (Beauchemin *et al.,* 2007 & Buddle *et al.*, 2011). Enteric methane (CH_4) is produced under anaerobic conditions in the rumen, by methanogenic *Archaea* that obtain energy by reducing carbon dioxide with hydrogen to form CH_4. These challenges have led research to establish strategies that can help to reduce methane production without compromising the animal performance. The studies have revealed that condensed tannins are capable of decreasing methane emissions by inhibiting methanogen's growth and indirectly by decreasing rumen fiber degradability hence, a reduced H_2 availability for CH_4 production

(Tavendale *et al.*, 2005). The *in vitro* tannins bioassay studies have revealed that tannins are capable of reducing methane production in ruminants. The bioassay involves incubation of plant samples with rumen fluid in the presence or absence of a tannin-binding agent such as polyethylene glycol (PEG). Polyethylene glycol has been used to inactivate tannins and thus neutralizes their negative effects on feed intake and digestibility in ruminants especially sheep, goats and cattle (Mlambo *et al.,* 2008). The variations in fermentation characteristics provide information on the potential biological effects of tannins in rumen fermentation. The tannins are evaluated *in situ* (without the need for extraction) and therefore the total tannin biological activity against a microbial population is measured.

2.6.3.4 Productivity of ruminants on tannin-rich diets

Some of tanniferous forage may be beneficial in ruminants, as they can improve utilisation of dietary protein, which may result in faster growth rates of live weight or wool, increased milk production, increased fertility, and improved animal welfare as well as health through prevention of bloat and lower worm load (Waghorn, 2008; Patra & Saxena, 2011). Mueller-Harvey (2006) indicted that despite the vast chemical variation of tannin structures, one common property is their ability to bind protein. Min (2003) noted that the condensed tannins bind proteins and other molecules firmly at near-neutral pH, such as in the rumen, but dissociate in the acidic pH of the abomasum, freeing protein for digestion. Dissociation of protein from condensed tannins, allows hydrolyzation of proteins by enzymes to amino acid. These amino acids add to the ones released during hydrolyzation of rumen microbial bodies which increases the flow into the duodenum. Moderate levels of condensed tannins in diets help on efficient utilization of nutrients such as nitrogen. Mueller-Harvey (2006) reported reduced excretion of urinary N on ruminants fed tannins containing diets and only slightly more faecal N. This could indicate that they absorb more of

the dietary amino acids from these tanniferous feeds than from iso-nitrogenous, non- tannin-containing feeds (Mueller-Harvey, 2006). Barry & McNabb (1999) reported that lamb grazing *Lotus corniculatus* had an increased wool growth by 12% without affecting rate of body growth.

2.7 Nutritional challenges of condensed tannins forage

Condensed tannins in plants serve as defense agents to deter herbivores as they are bitter (Hattas *et al.,* 2011). Plants with inductive mechanism can accumulate high concentration of this compound in response to herbivory. High levels of condensed tannins in feed can decrease feed consumption, ruminal degradation of protein, carbohydrate and a decrease in animal performance (Acero *et al.*, 2010). Condensed tannins can lead to reduced feed consumption. Barry & McNabb (1999) reported that high levels of condensed tannins in feeds such as in *Lotus pedunculatus* (75–100 g/kg DM) can depress voluntary feed intake and ruminal degradation of carbohydrate and depressed rates of body and wool growth in grazing sheep.

On the other hand, Mlambo *et al.* (2008) reported that reduced rumen protein degradability, limits the supply of rumen ammonia for microbial activity. This, in turn, negatively affects the utilization of poor quality cereal crop residues that are a major component of ruminant livestock diets in semi-arid areas. Nsahlai *et al.* (2011) also emphasized that tannins can also reduce the intake of food, depress the rate of breakdown of fibre, and protein leading to low N supply to microbes when diet does not meet rumen degradable N, hence reduce the efficacy of microbial protein production. McSweeney *et al.* (2001) stated that tannins complexes with lignocellulose reducing or preventing microbial digestion or by directly inhibiting cellulolytic microorganisms or both. Despite limited rumen degradation due to tannin complexing with protein and lignoncellulose, Kermauner (2008) also indicated that formation of such complexes can exacerbate

negative effects in the upper part of gastro-intestinal tract (GIT): reduces digestibility of nutrients and harms mucous membrane of small intestine, particularly when higher levels of tannins are consumed.

The complex interlinkage between condensed tannins, protein and carbohydrates, limit ruminal microbes nitrogen and energy for degradation of consumed feed leading to inefficient, depressed rate of degradation of fibrous feeds (Nsahlai *et al.* (2011). Barry & McNabb (1999) reported that high levels of condensed tannins in *Lotus pedunculatus* (95 and 106 g/kg DM) led to depression of ruminal degradation of readily fermentable carbohydrate (soluble sugar pectin) and hemicellulose, though it resulted in increased post-ruminal digestion.

2.8 Tannin toxicity in ruminant animals

Condensed tannin toxicity occurs when forage with tannins is consumed in large quantities. The cases of toxicity are rare, as Estell (2010) observed that ruminants survive plant secondary metabolites by cohesive behavioural and physiological strategies that involve both pre-ingestive (sensory) and post-ingestive processes. Behavioural mechanisms to cope with plant secondary metabolites include reduced ingestion, avoidance as they are able to make sophisticated choices (especially bitter compounds), vigilant sampling to attain familiarity with consequences, picking plant parts with lower levels, temporary intake cessation, altering feeding patterns and diet composition (Karban & Agrawal, 2002). Barry & McNabb (1999) reported that high levels of condensed tannins in *Lotus pedunculatus* (95 and 106 g/kg DM) which led to depression of rumen digestion of readily fermentable carbohydrate (soluble sugar pectin) and hemicellulose, although counteraction by increased post-ruminal digestion. Post ingestion is common in some browsers which secrete saliva rich in condensed tannin-binding proline. The tannins have higher affinity

to proline-rich protein, so it binds to tannins (Hagerman & Butler, 1981; Barry & McNabb, 1999). Through this mechanism herbivores are able to feed on tanniferous diets with reduced risk of toxicity.

2.9 Factors affecting chemical composition and condensed tannin levels

The processes of plant growth and biosynthesis of metabolites are greatly influenced by plant genetics and its environment. Studies have revealed that various biotic factors such as stage of growth, herbivory and abiotic which include soil characteristics (fertility, pH, and etc), temperature, light, ozone and amount of rainfall received that plants are exposed to during growth affects plant growth and its chemical composition (Kraus *et al.*, 2003). These factors contribute significantly to plant growth and biosynthesis of both primary metabolites (carbohydrates, amino acids, fatty acids) and secondary metabolites of plants such as phenolics, terpenes and alkaloids (Bourgaud *et al.*; 2001; Rispail *et al.*, 2005). Secondary plant metabolites are present in specialized cells that are not directly essential for basic photosynthetic or respiratory metabolism but are thought to be required for plant's survival in the environment (Lattanzio, 2013). They are structurally and chemically much more diverse than the primary metabolites.

2.9.1 Genetics

Genetics plays a crucial role in the response of plants to various environmental factors and contributes significantly to plant's performance. Some of browse plants are leguminous while others are not. Table 2.1 shows chemical composition of different browse plants leaves and the chemical components of different plants differs mainly due to genetic variation, although other cause of variation can be abiotic factors such as location of studies. Sattelmacher *et al.* (1994) indicated that genotypes may vary on effectiveness of nutrients utilization and uptaking of nutrients

from the soil (uptake efficiency). Sanon (2007) observed variation on concentration of different chemical components of *Acacia senegal, Guiera senegalensis* and *Pterocarpus lucens*, species. The level of crude protein (CP) was 114 (*G. senegalensis*), 157 (*P. lucens*) and 217 g/kg DM (*A. Senegal*). Their fiber components (NDF, ADF, and ADL) differed as *G. senegalensis* had significantly higher NDF, ADF, and ADL (604, 409 and 240 g/kg DM, respectively), than in the other two species, and the fiber fraction was significantly higher in *P. lucens* than in *A. senegal*. The apparent digestibility of CP in the browse leaves diets was 0.63 g and 0.64 for *A. senegal* and *P. lucens* leaves, respectively (Sanon, 2007). Islam *et al.* (2003) also investigated chemical composition of different varieties of *Pennisetum purpureum* (Arusha, Hybrid and Pusha) were fractionated botanically into leaf blade, leaf sheath, stem and head. They reported that all botanical fractions differed due to the effect of variety.

Table 2.1: Chemical composition of some browse plants

	Ash	OM	CP	NDF	ADF	ADL	DMD	P	Ca	Mg	Authors
Olea africanum	61.7	907.2	111.9	397.5	26.4	230.9	-	-	-	-	Gemeda & Hassen (2015)
Ziziphus mucronata	96.9	840.4	243.3	339.2	27.2e	101.4	-	-	-	-	
Ziziphus mucronata	2.43	-	7.08	54.6	41.9	-	34.11	-	-	-	Aganga & Mosase (2001)
Coddia rudis	-	913.0	10.625	32.0	-	-	-	0.14	1.3	0.27	van Lieverloo *et al.* (2009)
Grewia robusta	-	91.3	15.625	35.7	-	-	-	0.13	1.4	0.23	
Ehretia rigida	-	86.5	20.625	345	-	-	-	0.20	1.6	0.45	
Phyllanthus verrucosus	-	92.8	13.75	161	-	-	-	0.26	1.2	0.25	

2.9.2 Plant height/harvesting stage

Concentration and properties of plants metabolites change due to changing conditions as the plant matures, so it is crucial to determine the stage of growth when the nutritional value is at a peak. Fernandes *et al.* (2013) reported that the concentration level of five different substances varied according to harvesting time, suggesting that the biosynthesis of polar metabolites of *Calendula officinalis* were affected by harvesting time. Lees *et al.* (1994) also measuring effects of temperature stress on level of condensed tannins in *Lotus uliginosus* Schkur every 3 weeks and reported significant levels after 14 days. Achakzai *et al.* (2009) reported the distribution and concentration levels of different plant metabolites in plant parts. They reported that leaves and stems of all plant species in their study contained alkaloids and their concentration in leaves was relatively higher than in the stem of the same plant species. The accumulation of alkaloids in young plant parts generally is greater as compared to older plants. This could be due to inductive mechanism in which plant response to factors such as herbivory of nutritious, tender leaves so accumulation of bitter secondary metabolites serves to protect and scare away herbivores. Suksombat & Buakeeree (2006) and Lounglawan *et al.* (2014) reported that increasing the harvesting interval (i.e. advancing age of maturity) increased dry matter and nutrient variation significantly. It also increased the cell wall constituents including crude fiber, ADF, NDF and ADL percent in the plant. However, crude protein and ash percent markedly declined as the cutting interval increased. Azuhnwi *et al.* (2011) reported that harvesting period had a significant effect on most compositional variables as well as on digestibility and gas production in *Juniperus communis* trees. In their study, higher crude protein and condensed tannin concentrations were found in the second harvest compared with the first harvest. Petrussa *et al.* (2013) reported that flavonoids, such as condensed tannins, are widely distributed according to the plant species, organ, developmental stage and growth conditions. Very little is known about the

distribution of secondary metabolites in terms of plant age or developmental stage (Achakzai *et al.*, 2009). Berard *et al.* (2011) observed that condensed tannin concentration in forage legume species was higher in the mature samples than the vegetative samples. So determining concentration of chemical components at different stages of growth in different plant parts can help to establish the period and parts where concentration of chemical components is high.

Estiarte *et al.* (1994) reported that the biosynthesis of phenolics is controlled by several abiotic and biotic environmental factors and depends on how these factors affect growth and photosynthesis. Change in chemical composition of plant is greatly affected by different biotic and abiotic factors. These factors stimulate mechanical ways of protecting itself so that it maintains normal status. However, chemical composition of browse can be greatly affected stage of growth.

2.9.3 Herbivory/defoliation

Plants have developed various defense strategies against different environmental factors, including herbivores. The plant defense mechanism can be inductive in those plants that react to external stimuli such as herbivory by producing larger quantities of antinutritional compounds to protect themselves. The defense mechanism can also be constitutive in those plants that produce defense compounds because of their genetic make-up (Frost *et al.,* 2008). Studies have revealed that plants have biochemical and physical response to herbivory to scare herbivores. Some of the strategies include secretion of plant secondary metabolites such as phenols, condensed tannins, and production of thorns.

Defoliation has been identified as one of the factors that lead to increased production of tannins in plants. The production of tannins can be accelerated by abiotic stress such as herbivory. Accumulation of secondary metabolites is generally a defense mechanism of plants which protect the plants from mechanical damages from herbivores. Skarpe & Hester (2008) and Ashok & Upadhyaya (2012) indicated that tannins are astringent and bitter causing dry and pucker feelings in the mouth. It is noted that tannins, are assumed to function as chemical defense that contributes to the herbivore-avoidance strategies of plants and defoliation may induce production of tannins in woody plants (Ward & Young, 2002; Wessels *et al.,* 2007). Condensed tannins have specific taste and smell that discourages herbivores, insects and humans from eating the plants hence sometimes called anti-grazing factors. Roitto *et al.* (2009) reported that phenolic compounds often accumulate in foliar tissues of deciduous woody plants in response to previous insect defoliation, but similar responses have been observed infrequently in evergreen conifers.

Studies such as by Maeda & Dudareva (2012) revealed that wounding also induces mRNA encoding of phenylalanine ammonia- lyase (EC 4.3.1.5). This enzyme catalyzes the synthesis of phenylalanine, a common precursor of numerous phenolic compounds, which include flavonoids, condensed tannins, lignans, lignin, and phenylpropanoid/benzenoid volatiles that play crucial roles in plant growth, development, reproduction, defense, and environmental responses (Maeda & Dudareva, 2012). The specific wound-induced protein synthesis is preceded by an increase in the mRNA encoding this enzyme. The induced mRNA of potato tuber, leaf, and stem tissue is translated into a precursor polypeptide that is recognized by antibodies raised against the mature enzyme from tuber plastids.

2.9.4 Altitude

Elevation has a significant role in the health and growth of plants as it may influence sunlight (type and amount) that plants receive, the amount of water that plants can absorb and the nutrients that are available in the soil. Plants respond in varying ways to different elevations they grow in as certain plants grow very well at high altitudes, whereas others can only grow in middle or lower elevations. Owuor *et al.* (1990) reported that differences in altitude within a radius of 10 km led to significant alteration in all quality constituents of crush, tear and curl black clonal teas. They noticed that synthesis of group I volatile flavour compounds was increasing as altitude decreased while group II VFC and flavour index increased as altitude increased. This could indicate the inductive mechanism that led production of some chemical substances and change in chemical composition to enable the plant to cope with changing altitude. Gale (2004) also emphasized that when considering the effect of altitude on whole plant physiology and ecology, it is crucial that all environmental factors should be taken into consideration, not only leaf gas exchange under saturating light and otherwise optimal conditions. Körner *et al.* (1988) noticed that an increase in altitude led to increase in 13C isotopes of the leaves of C3 plants. Mountousis *et al.* (2006) reported that altitude had a very significant influence on ash, ether extracts, crude fibre and crude protein of plant leaves. Martz *et al.* (2009) reported that the level of soluble phenolic (proanthocyanidins, and flavonols in northern latitudes) and terpenoid (monoterpenoids) in juniper needles increased with latitude and altitude.

2.9.5 Soil moisture

Soil is the growth medium for plants providing different nutrients that affect rate of growth and biosynthesis of plant metabolites. Plants grown on less fertile soil are expected to produce high level of condensed tannin due to nutrient stress. Kraus *et al.* (2004) confirmed that changes in soil

nutrient supply have a great influence on secondary plant compound production.

Water is also one of the growth environment factors that can lead to plant stress. Krasensky & Jonak (2012) reported that responses of plants to stress are dynamic and involve complex cross-talk between different regulatory levels, including adjustment of metabolism and gene expression for physiological and morphological adaptation. Drought stress is one of the most significant abiotic stresses that affect plant growth and development. Ramakrishna & Ravishankar (2011) explained that drought stresses lead to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm to vacuoles. The inductive mechanisms enable plants to produce secondary metabolites in response to stress (Said-Al *et al.*, 2009). They indicated that water stress in plants affects many metabolic processes and the extent of its effects depends on drought severity. Drought often causes oxidative stress and was reported to show an increase in the amounts of flavonoids and phenolic acids in willow leaves. Some of the secondary metabolites produced are flavonoids like condensed tannins, which are widespread throughout the plant kingdom, presenting diverse biological and biochemical activities. Flavonoids are thought to have protective functions during drought stress (Ramakrishna & Ravishankar, 2011). Becerra-Moreno (2015) observed that water stress activated the primary and secondary metabolism of carrots, which favoured accumulation of lignin. On the other hand Ojeda *et al.* (2002) investigated the effects of various levels of water deficit on the biosynthesis of and concentration of skin phenolic compounds (flavan-3-ols, anthocyanins, and flavonols) on growing Shiraz berries. Ojeda *et al.* (2002) reported high levels of flavonols at medium and strong water deficiency than at control. They reported that biosynthesis of total tannins was low in the early water deficit samples; while biosynthesis of proanthocyanins and anthocyanins increased only at maturity, the late water deficit treatment. In all cases, water deficit increased the degree of tannin

polymerization, revealing inductive mechanism in plants. Table 2.1 also revealed varying chemical composition of different browse plant leaves, harvested from different areas which could be having different soil type, moisture content, fertility and structure.

Insufficient water or drought can result in accelerated generation of reactive oxygen species (ROS) in plants due to disturbance of cellular homeostasis. Shortage of water, especially under high light intensity or in combination with other stresses, disrupt photosynthesis and increase photorespiration, changing the normal homeostasis of cells and lead to an increase in production of ROS (Miller *et al.*, 2010). Reactive oxygen species can serve in response to abiotic stresses functioning as toxic by-products of stress metabolism, as well as vital signal transduction molecules. These ROS are extremely harmful to organisms at high concentrations (Sharma *et al.*, 2012). High levels of ROS can lead to excess defense mechanisms, a cell is said to be in a state of oxidative stress. Hasanuzzaman *et al.* (2013) showed that ascorbate, glutathione (GSH), carotenoids, tocopherols, and phenolics serve as potent non-enzymic antioxidants within the cell to combat oxidative stress induced by various environmental stresses.

2.9.6 Soil fertility

Shortage and inconsistent supply of nutrients has been identified as one of the factors that can stress the plant and affect growth and biosynthesis of plant metabolites. Kraus *et al.* (2004) reported that decreasing levels of fertilizer led to an increase in the concentrations of foliar total phenols and condensed tannins by 1.2-2.0 times. These confirm that nutrient stress can lead to production of condensed tannins. Most investigations such as by Kraus *et al.* (2004) found that phenolic and tannin concentrations increased when nutrient availability decreased and/or carbon availability increased. Plants growing under unfavourable conditions often contain high

concentrations of foliar tannins and other phenolics.

Sariyidiz & Anderson (2005) reported that location, soil fertility and tree species had effects on the chemical composition of green leaves and leaf litters of sweet chestnut (*Castanea sativa*), oak (*Quercus robur*) and beech (*Fagus sylvatica*). These tree leaves were harvested from 26 sites of different soil fertility (low and high) had varying chemical components between and within tree species. Beech leaves grown on high fertile soil had lower levels of ADF, lignin and cellulose than on low fertile soils, whereas oak and chestnut leaves had higher ADF, lignin and cellulose on highly fertile soils.

2.9.7 Temperature

Temperature is one of the vital environmental factors that affect plant growth and its chemical composition. Hasanuzzaman *et al.* (2013) indicated that stress due to temperature especially high temperature, is one of the major environmental stresses that limits plant growth, metabolism, and productivity worldwide. The numerous biochemical reactions that are involved in plant growth and development are sensitive to temperature. Change in temperature normally lead to a shift in plant metabolism, affecting biosynthesis of both primary and secondary plant metabolites. Zobayed *et al.* (2005) reported that temperature can cause many physiological, biochemical and molecular changes in plant metabolism such as protein denaturation, lipid liquefaction or perturbation of membrane integrity, which in turn affect secondary plant metabolites. Bita & Gerats (2013) indicated that the susceptibility to high temperatures in plants varies with the stage of plant development and the impacts depend on species and genotype. Plants display various cellular and metabolic responses for survival when their growth environmental temperature is at least 5°C above their optimal growing conditions (Bita & Gerats, 2013). The response include a

change in the organization of cellular structures, such as organelles and the cytoskeleton, and membrane functions including reduced biosynthesis of normal proteins and the accelerated transcription and translation of heat shock proteins. High temperature also accelerates the rate of cell lignification (Van soest, 1988). Lees *et al.* (1994) reported that *Lotus uliginosus* (Schkur) matured more quickly and had higher levels of condensed tannins when exposed to higher temperature (30^{O}C) than plants of the same clone grown at normal temperature (20 ^{O}C).

2.9.8 Ozone

Ozone is a vital component of the atmosphere, despite existing in trace amounts it has some impact on plant growth and composition (Iriti & Faoro, 2009). There are two varying pools of ozone, being, the stratosphere and troposphere. The stratosphere ozone is at a higher atmosphere, with range of about 15 to 40 km in which forms a layer that absorbs the harmful UV-B and UV-C radiations. It is considered beneficial ozone but it has been reduced by emission of ozone depleting chemicals and gases. Iriti & Faoro (2009) explained that at the lower part of the atmosphere, approximately from the earth surface to 10 - 12 km in altitude (troposphere), the layer where the climatic conditions originate, and temperature decreases with elevation. Lowering of temperature as the altitude increases may end up stressing the tree which can induce production of stress coping mechanisms such as production of plant secondary metabolites. Tropospheric ozone is an oxidant constituent of the photochemical smog and it is considered a pollutant. This ozone is a secondary pollutant and is the end product of reactions among primary pollutants, released directly into the air (mainly nitric oxides, sulphur oxides, carbon oxides and hydrocarbons), catalyzed by sunlight (Crutzen & Lelieveld, 2001) is responsible for impaired plant growth and lead to a decline on yields. Tropospheric ozone production is caused by photochemical reactions of carbon monoxide (CO), methane (CH_4), and other hydrocarbons in

the presence of NOx (NO + NO_2) reactions, involving NO, HO_2, or OH (Felzer *et al.,* 2007). This component of the atmosphere has been reported by some as hazardous gaseous pollutant (Rozpadek *et al.*, 2015) that have negative impacts on growth and chemistry. Others such as Crutzen & Lelieveld (2001) revealed a positive role of near ambient concentrations of ozone on plant growth to have both positive and negative impact on plant growth. Nevertheless previous reports showed that doses of up to 150 $\mu g/m^3$ led to increases on the productivity of plants such as *Phaseolus vulgaris*, a natural grassland species and trees (Rozpadek *et al.,* 2015).

Felzer *et al.* (2007) emphasized that exposing vegetation to ozone (tropospheric) may lead to reduction of photosynthesis, growth, and other plant functions. Rozpadek *et al.* (2015) indicated that ozone (O3) is a model abiotic elicitor of reactive oxygen species (ROS) in plant cells. It enters the leaves through open stomata and due to its high reactivity immediately reacts with components of the apo-plastic space generating various ROS and activating detoxification, including enzymatic and non-enzymatic antioxidants and other defense mechanisms. Felzer *et al.* (2007) also noted that ozone affects physiological activity such as a declined rate of photosynthesis, while increased turnover of antioxidant systems damage to reproductive processes, increased dark respiration, lower carbon transport to roots and lead to reduced decomposition. The reaction of plants to ozone exposure seems to differ with species. Gaseous ozone diffuses into plants through stomata, then dissolves in water. It negatively affects the vegetation by directly damaging the cells (especially palisade mesophyll cells) upon entry/exit through stomates. Plants with an inductive mechanism respond to the damage of the cells by producing secondary metabolites such as phenolics for defense, while those with a constitutive mechanism will ensure that defense mechanism is always there.

2.9.9 Carbon dioxide

Carbon dioxide plays a crucial role in food production during photosynthesis in green plants, but high levels of this gas can impair this process. Felzer *et al.* (2007) observed that increased amount of internal carbon dioxide due to ozone lead to the closure of the stomata due to decreased photosynthetic activities caused by the ozone. Myers *et al.* (2014) grew and exposed 18 cultivars of rice (*Oryza sativa*,), 8 cultivars of wheat (*Triticum aestivum*), 2 cultivars of maize (*Zea mays*), 7 cultivars of soybeans (*Glycine max,),* 5 cultivars of field peas (*Pisum sativum*) and sorghum (*Sorghum bicolor*) to ambient (364–386 ppm) and elevated (546-586 ppm) levels of carbon dioxide in free air carbon dioxide experiments (FACE). They reported that nutrient levels of the edible parts of these crops declined on elevated carbon dioxide especially their zinc, iron, and sometimes protein concentrations. The influence of high carbon dioxide on legumes differed among plant functional types, with C3 grasses and legumes consistently affected and C4 plants less so. They observed that the effects on nutrient contents differed among various cultivars of the same crop species (Dietterich *et al.,* 2015).

Several studies have reported that a rapid short-term doubling of current atmospheric carbon dioxide concentrations leads to inhibition of respiration of mitochondria and plant tissues by 15–20 % (Gonzalez-Meler *et al.*, 2004). Despite the direct impact of carbon dioxide on respiratory enzymes that has been reported, the degree of the direct inhibition of intact tissue respiration by high carbon dioxide has now been revealed to be described by measurement artefacts (Gonzalez-Meler & Jahnke, 2001), diminishing the effect that such declines in respiration rate may have on plant growth and the global carbon cycle. Research has revealed that high level of carbon dioxide affects respiration by impeding oxygen uptake of isolated mitochondria and the activity of mitochondrial enzymes under some conditions (Gonzalez-Meler *et al.*, 2004).

2.9.10 Light

Light plays an integral role on plant growth, morphology and chemical composition especially in green leaves with chlorophyll that traps the sunlight for photosynthesis (Paulilo *et al.,* 2010). Onoda *et al.* (2008) also emphasized that light accessibilities have significant effects on leaf morphology and chemistry, as its availability, intensity and duration of exposure can alter them. Tree leaves under sunlight for a longer period will have more photosynthesis than those that have shorter period, as light energy facilitates photosynthesis. As the plant grows, old leaves tend to be shaded by new growth and leaves tend to receive varying levels of light which may alter the chemical composition of leaves. Onoda *et al.* (2008) indicated that plants typically respond to shading by producing leaves with less mass per unit area (LMA), which enables greater light capture per unit mass. Rozpadek *et al.* (2015) indicated that plant growth environment such as light quality and quantity are some of the main factors influencing the amount and composition of essential phytochemicals. Shortage of light and its intensity can stress the tree leaves and may lead to production of secondary metabolites. Paulilo *et al.* (2010) indicated that the key ecological purpose of the secondary metabolites is to enable plant species to cope with environmental stresses. Onoda *et al.* (2008) reported that light during growth had a significant effects on the mechanical properties of leaves. The strength of leaves exposed to sunlight was significantly higher than shaded leaves. High strength with light accessibility could be accredited to a concomitant increase in LMA. However, shaded leaves were significantly stronger relative to their mass (higher Fmax/ML), which partly compensated for their lower LMA. This greater Fmax/ML of shaded leaves could have been due to greater deposition of dry mass to cell walls material and cell-wall strength.

2.10 Techniques used for determining the nutritional value of feeds

2.10.1 Estimation of chemical composition of browse plants

Efficient utilization of scarce feed requires knowledge and understanding of their chemical content as well as the interaction and availability of those nutrients to the animal. This will ensure proper diet formulation to meet the nutritional requirements of the animals. Many researchers including Holechek *et al.* (1990) and Van Soest (1993) emphasized that high lignin and condensed tannins in browse plants negatively affects ruminal degradation of protein and cell wall. Sanon (2007) also emphasized the negative relationship between lignin concentration and cell wall on DM digestibility as it acts as a physical barrier impeding microbial enzymes to penetrate and digest cell contents. Therefore, information on the NDF, ADF, lignin and tannin content of browse tree leaves is vital for proper animal feeding (Sanon, 2007). There are traditional and conventional procedures that have been used lately for analyses of both primary and secondary plant metabolites. They include proximate analysis which seeks to determine cell wall or fibre (NDF, ADF, ADL, cellulose and hemicellulose), following the procedure by van Soest *et al.* (1991), crude protein in the samples is determined using the Standard macro-Kjeldahl method used for determining crude protein in feed samples. Different plants contain varying chemical substances, at different levels, in the case of browse plants there are secondary metabolites, such as phenolics and tannins, which play crucial roles in animal nutrition. Analysis of soluble phenolics (SPh) can be done using Folin- Ciocalteau reagent and gallic acid as a standard according to Makkar (2003). The procedure by Terrill *et al.* (1992) can also be used for determination of insoluble phenolics.

2.10.2 Procedures for determination of degradability of ruminants feed

2.10.2.1 *In sacco* technique

Determination of chemical composition of feedstuff is vital for understanding the nutritional potential of the feed, but it is not enough as interaction of some of the chemicals contained in that particular feed can inhibit ruminal degradation, extraction and use of the other (D'Mello, 1992). This necessitates further analysis to estimate ruminal degradation of feeds consumed by the animal. Such studies include *in sacco* and *in vitro* ruminal degradation techniques. Rumen degradability of the feed is normally estimated by use of the *in sacco* procedure also known as the nylon bag technique (Ørskov *et al.,* 1980; Jančík *et al.,* 2010). This method is used for the determination of the rate and extent of degradation of feedstuffs in the rumen at different incubation periods and can also be used to screen feeds at the initial stage of assessing their nutritive values (Ørskov & McDonald, 1979). The *in sacco* method is used for determining the degradation parameters of a whole spectrum of nutrients. Quin *et al.* (1938) introduced the *in sacco* technique (nylon bag or in situ technique) and studied ruminal degradation of ruminant feeds through incubation of weighed feed samples into silk bags and inserted in rumen through a cannula. The silk bags were substituted with nylon bags (Rodriguez, 1968). The nylon bags have small pores which allow microbes to access the incubated feed sample. The technique involves incubation of feed samples at the same time and withdrawn at different periods (sequential withdrawal) or incubation at different periods and withdrawn at same time (sequential incubation). The feed samples are exposed to rumen microbes at fixed times (after 0, 2, 4, 8, 12, 24, 36, 48, 72 and 144 h of incubation) to determine the progressive disappearance of feed from the bags, as well as estimating both rate and extent of feed degradation (Mehrez & Ørskov, 1977; Kitessa *et al.,* 1999). The nylon bag technique has been largely used to evaluate rumen degradability of feeds over *in vivo* digestibility (Gosselink *et al.,* 2004; Damiran *et al.,*

2008) and it was found to predict the *in vivo* digestibility well (Fonseca *et al.*, 1998).

In sacco procedure appears to be poorly standardized and plagued by low repeatability and reproducibility (Madsen & Hvelplund, 1994). The technique has a main challenge of variation of porosity of the bags. The proper bag porosity must allow influx of rumen fluid and, efflux of degraded feed particles, and retention of feed particles not yet fermented (Mehrez & Ørskov, 1977). Bags with too small (< 35 µm) porosity can lead to sorting of microbial population by size (Meyer & Mackie, 1986) while degraded particles maybe retained in the bags, with a consequent occlusion of bag pores and accumulation of gas inside the bags (Van Soest, 1984). On the other hand, too large porosity (50-60 µm) can lead to the escape of undigested feed particles (Vanzant *et al.,* 1998). Vanzant *et al.* (1998) recommended a pore size from 20 to 60 µm. Another important factor to consider was size of the feed particles to be incubated as large particle size will lead to slow degradation while fine ones will easily be degraded in short period of time. Hence, Nocek (1985) suggested that the grinding must reproduce the effects of rumination and that feed sample size should be as similar as possible to the size of ruminated feed. Generally, a coarse size (4-5 mm) is related to lower and slower degradation rates, whereas a fine grinding size (< 2-3 mm) is supposed to facilitate the escape and the loss of feed particles from the bag (Michalet-Doreau & Ould-Bah, 1992). Nocek (1988) suggested 2 mm screen size for high-protein feeds, and a 5 mm-screen for more fibrous feeds (grains, by-products and roughages). Stern *et al.* (1997) indicated that *in sacco* technique has been criticized heavily by public opinion for use of cannulated animals which led to ethical and moral issues about animal welfare. This technique is also costly, laborious, time consuming with limited analytical capacity necessitating development of an alternative method or Daisy incubation procedure.

2.10.2.2 *In vitro* dry matter degradability

In vitro ruminal degradability or daisy incubation (ANKOM Technology®, Macedon, NY, USA) was invented as an alternative laboratory method that mimics rumen degradation of feeds. This method is cheaper, less laborious, cost effective, is more efficient and most suitable for large-scale evaluation of ruminant feeds, as it allows simultaneously incubation of up to 100 feed samples. The *in vitro* technique can be classified on determination of degradability of feeds (Tilley & Terry, 1963) and measurement of gas produced (Menke *et al.,* 1979).

Tagliapietra *et al.* (2008), compared Daisy^{II} and a conventional batch culture, and noticed that the digestibility values obtained with Daisy^{II} were constantly lower and less repeatable, and attributed this result to a barrier effect exerted by the bags, which changed the normal income and outcome of rumen fluid and feed particles. On verifying the sample size to be incubated, several researchers (Holden, 1999; Mabjeesh *et al.,* 2000; Tagliapietra *et al.*, 2008) incubated 0.50 g feed sample/bag, while Spanghero *et al.* (2003) preferred 0.25 g. Damiran *et al.* (2008) also compared F57 filter bags and standard nylon bags, and the two feed sample sizes generally used (0.25 and 0.50 g feed sample/filter bag): They reported that digestibility values of the two types of bag had high correlation, while digestibility values of 0.50 g feed sample/filter bag were better correlated with those obtained with the nylon bag technique. This shows that 0.5 g of sample incubated in F57 filter bags in Daisy II incubator is the best procedure to mimic ruminal degradability of feeds and will be used in determining the degradability of browse plant leaves in this study.

Browse plants are known to contain plant secondary metabolites such as phenolics and condensed tannins which form complexes with protein and fibre. The ruminal degradability researches

have shown that these complexes delay and reduce the rate and extent of degradation of the browse plant leaves. Barry & McNabb (1999) observed that high levels of condensed tannins (95 and 106 g/kg DM) in *Lotus pedunculatus* reduced rumen digestion of readily fermentable carbohydrates. Isah *et al.* (2012) determined dry matter degradability for browse plant leaves (*Azadirachta indica, Ficus exasperate, Synedrella nodiflora* and *Boerhavia diffusa*) at 24 and 48 h of incubation. They reported a decline in the rate of dry matter degradability (DMD) of these browse plant leaves after 24 hours of incubation which could have been due to plant secondary metabolites. The findings concurred with several studies (Lila *et al.,* 2003; Patra *et al.,* 2006; Salem, 2006). Patra *et al.* (2006) observed a decline on *A. indica's* leaves and suggested that the astringent factor might have reduced the protozoa population leading to decline on the *in vitro* dry matter degradability (IVDMD) between 24 and 48 hours of incubation. Another research by Ferri *et al.* (2004) emphasized that IVDMD rate of leaves *of A. indica* was reduced between 24 and 48 hours post incubation which could be due to higher levels of NDF and lignin. These findings by different researchers were used as a guide on the withdrawal periods on this current study.

2.10.2.2 Determination of nitrogen buffer solubility, in vitro ruminal nitrogen degradability

The ruminal protein degradation is a vital procedure for assessment of the quality of protein in any feed material edible to ruminant animals (Mnisi, 2015). Thus, forage intake is mainly influenced by the rate of the forage degradation by ruminal microbes, the rumen size, the forage digestibility and passage rate through the gut (McDonald *et al.*, 2002). Sanon (2007) advised that establishing the digestibility of browse fodders is crucial as it helps in estimation of nutrients available for animal nutrition. Since *in vivo* digestibility and voluntary intake experiments with animals are laborious, time consuming, expensive and require large feed quantities (Karsli &

Russell, 2002) *in vitro* ruminal protein degradation characteristics of browse species can represent an accurate measure of the quality of protein for ruminant animals (Edwards *et al.,* 2012). Buffer solubility of nitrogen serves to estimate the amount of rumen degradable N in the feed samples following the modified Licitra *et al.* (1999) procedure.

In most cases the nutritional value of browse tree leaves normally vary due to the genetic variation of plant species, edaphic, harvest stage and height as well as processing of leaves (Beever & Mould, 2000). Increasing knowledge of ruminal degradation of buffer soluble proteins for browse species can be vital for animal productivity (Hedqvist, 2004). Correlation between buffer solubility of a protein and rumen degradability has not been fully demonstrated, but has been shown to depend, largely, on type of feed and type of protein, among other factors (Aufrere *et al.,* 1991). Stern & Satter (1984) highlighted that N solubility has poor predictive ability to measure bypass protein amongst feeds.

2.10.3 Near infrared reflectance spectroscopy

Researchers have been inventing and perfecting procedures and techniques that assist efficient analyses of nutritional value of diet ingredients. There are traditional and conventional procedures that have been used lately for feed analyzes. They include proximate analysis which seeks to determine cell wall or fibre (neutral detergent fibre, acid detergent fibre, lignin, cellulose and hemicellulose), ether extracts, crude protein in the samples is determined using the Standard macro-Kjeldahl method used for determining crude protein in feed samples.

Near infrared reflectance spectroscopy (NIRS) uses the light, which produces spectrum, the variation in energy is reflected by differing spectra as a series of absorptions at different

wavelengths (Cozzolino & Moron, 2004). Plants contain varying levels of different chemical substances, hence chemical bonds of different strength, showing that the different amounts of energy are needed for the bond vibrations to move from one level to the next in different forage samples. These absorptions are due to interactions with the fundamental vibrations of the chemical bonds associated with the atoms of the groups. The chemical bonds include C–H, N–H and O–H bonds, which are the primary constituents of organic molecules as well as nitrogen, cellulose and lignin in forages (Morris, 1989). Landau *et al.* (2006) explained that NIRS calibration involves development of mathematical models that correlate wet chemistry results with the spectra produced from absorption of light at different wavelengths in the near infrared region (1200 to 2500 nm), determined by reflectance (Deaville & Flinn, 2000). Diffused reflection contains information that identifies chemical bonds within the sample, including -OH, -SH, -NH and - CH bonds, which enables identification of protein, structural fibre, sugars, lipid and ash and their concentration (Dryden, 2003; Ramirez *et al.*, 2015). The precision of prediction of NIRS depends heavily on obtaining a calibration set which represents the variation in the main population, accurate laboratory analyses and the application of the best mathematical procedures (Swart, 2012).

2.10.3.1 Advantages of NIRS

Near infrared reflectance spectroscopy is one of the latest technologies that is most efficient (cost effective and fast), accurate and environmentally friendly (De Boever *et al.,* 1995) feed analysis procedure. This technique provides an alternative, appropriate procedure for assessment of proximate analysis and chemical composition including phenols in the feed material (Martin *et al.,* 1989) without the need for laborious laboratory analyses. The technique can also be used for determining degradability of feed once it has been calibrated which will eliminate laborious

procedure of cannulating animals and *in sacco* degradability. The rapidity of this method can assist on fast analysis of nutritional value of browse plants. Browse plants plays crucial role in livestock feeding, especially during dry seasons when the quantity and quality of forage has declined, though it has received less research attention.

2.10.3.2 Limitations of near infrared reflectance spectroscopy

The main challenge of use of NIRS is the fact that it requires independent data set to validate the results obtained from calibrated samples (Landau *et al.*, 2006). The need for an extensive database against which the instrument is calibrated and the importance of good wet chemistry backup to NIRS are emphasised, together with some of the diverse uses for NIRS in research and agriculture (Corson *et al.,* 1999). De Boever *et al.* (1997) indicated that NIRS is very sensitive to chemical and physical nature of samples, hence the need for more samples for chemical and *in vitro* analysis. Studies by De Boever *et al.* (2004) showed that prediction of rumen degradation characteristics of rumen degradability and protein quality of grassland products from a small number of samples with NIRS gave erroneous cross-validation indicating clearly that it was less accurate than with laboratory measurements. Foley *et al.* (1998) emphasized that the quality of the predictions made by NIRS depend mainly on the accuracy and precision of the reference values for calibration data set.

2.11 Summary

Despite the crucial role browse plants play in livestock feeding, most of the browse plants in this study have not been nutritionally characterized. Considering the fact that growth environment is one of the factors that can affect chemical composition of plants, there is the need to investigate them. Genetic variation of browse plant trees enables them to respond to various biotic and

abiotic factors in a peculiar manner as some plants will activate inductive while others activate constitutive mechanism. Inductive mechanisms allow the plants to alter its growth and chemical reaction such as secondary plant metabolites like phenolics and condensed tannins, in response to a particular factor that interferes with them. Other plants have constitutive mechanism that do not respond to stressing factors, that is the chemical reactions are always present in such tree plants.

Biotic and abiotic growth conditions of plants necessitates determination of chemical composition of plant parts such as leaves, at different harvesting heights to establish changes that occurred during growth. Browse plants leaves have high nutritional value which is sustained during dry period than grasses. It is also crucial to determine how those chemical changes affect utilization of plant chemical constituents at different harvesting heights, as interaction of plant chemical constituent can affect digestibility and availability of other nutrients. The complex formed by condensed tannins and proteins in the neutral pH may depress microbial population due to low supply of substrate leading to reduced degradation of feeds. Conventional methods are expensive, time consuming, laborious, uses reagents that can be toxic hence the need for alternative procedure that can be more efficient. One of the techniques that has been reported to be efficient (cheap, fast) and environmentally friendly is the near infrared reflectance spectroscopy. Since it is fast, more tree leaves can be analysed and provide their nutritional value to relevant stakeholders. The gathered information is crucial to the farming community as it will guide farmers on feeding of different classes of their animals and ensure efficient utilization of feeding.

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3 CHAPTER THREE: CHEMICAL CHARACTERIZATION OF BROWSE LEAVES HARVESTED FROM DIFFERENT PLANT HEIGHTS

Abstract

The purpose of this study was to investigate the influence of harvesting heights, [browsable (<1.5 m) and non-browsable (>1.5 m)], on the chemical composition of leaves from ten browse tree species. Leaves from trees (*Maytenus capitata, Olea africana, Coddia rudis, Carissa macrocarpa, Rhus refracta, Ziziphus mucronata, Boscia oliedes, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida*) growing in Soxhada communual grazing area of the Eastern Cape Province, South Africa were studied. The leaves were dried at 60 °C until constant weight, ground and analysed for dry matter (DM), proximate components, soluble phenolics, condensed tannins and minerals. Plant species as well as interaction between plant species and harvesting height had a significant ($P < 0.05$) influence on total N, total phenolic content, while harvesting height did not influence these substances. *Ziziphus mucronata* (27.0 g/kg N) and *G. robusta* (22.7 g/kg N), had the highest total N at non-browsable height, 25.1 and 21.4 g/kg N respectively at browsable height, while soluble phenols ranged between 553.2 µg TAEQ /kg DM for *B. oliedes* and 744.1 µg TAEQ /kg DM for *R. refracta.* The leaves contained moderate to high level of CP which ranged from 81.5 g/kg DM for *Carissa macrocarpa* to 168.6 g/kg DM for *Z. mucronata* at both heights and it was enough to meet CP levels required by microbes in the rumen, making them potential sources of supplementary protein during the dry seasons. *Ziziphus mucronata* and *G. robusta* had the highest CP at both browsable and non- browsable heights, respectively, and moderate CTs, crucial for increasing bypass protein and possible control of internal parasites. Tree species and harvesting height significantly affected condensed tannin (CTs) content where samples harvested from the non-browsable height had higher (0.61 $AU_{550\ nm/200\ mg}$) levels than

those harvested at browsable height (0.55 $AU_{550\ nm/200\ mg}$). The concentration of CTs ranged from 0.31 $AU_{550\ nm/200\ mg}$ in *P. vessucosus* to 1.04 $AU_{550\ nm/200\ mg}$ in *C. macrocarpa* leaves. There was no significant interaction between tree species and harvesting height on both macro and micro-minerals, but they were only influenced ($P<0.05$) by browse species. Phosphorus content of the leaves ranged between 0.6 to 2.9 mg/kg DM while Ca ranged from 8.7 to 24.3 mg/kg DM. The different browse leaves contained high levels of fibre, moderate to high protein, as well as moderate level of phenolics and minerals, suggesting that they have the potential to provide supplementary nutrients for ruminants during the dry seasons.

Key words: Browse plant leaves, harvesting heights, chemical composition, phenolics, condensed tannins

3.1 Introduction

Browse plants play an integral role as a source of nutrients in arid and semi-arid regions of Africa. Mlambo *et al.* (2008) & Tefera *et al.* (2008) indicated that browse plants can serve as the alternative and cheapest sources of nutritious feed, as they tend to maintain their chemical composition better than rangeland grasses and can be utilized to supplement ruminants during the prolonged dry seasons. In the Eastern Cape Province of South Africa, some of the common browse plants that have a potential to provide supplemental nutrients include; *Maytenus capitata, Olea africana, Coddia rudis, Carissa macrocarpa, Rhus refracta, Ziziphus mucronata, Boscia oliedes, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida.* Despite their pivotal role they play in animal nutrition, they have received little attention.

The knowledge and understanding of chemical composition is crucial in animal nutrition as they assist farmers to efficiently provide diet that will meet the nutritional requirements of their animals. The concentration of chemicals in different plant parts varies as the plant matures as they are exposed to various biotic and abiotic factors that affect their chemical composition. Makkar *et al.* (2003) indicated that these factors tend to stress the plants, stimulating biosynthesis of different levels of various secondary plant metabolites like phenolics, tannins etc. that act as defense mechanisms. Plants can have genes for either constitutive or inductive mechanism that they use mainly in their defensive strategy, which involves production of plant secondary metabolites that assist on defense against herbivores (Frost *et al.*, 2008). Therefore chemical composition of plants is expected to vary as plants grow due to biotic (genetics, herbivory etc.) and abiotic (soil moisture, fertility, temperature etc.) factors (Van Soest, 1982). Suksombat & Buakeeree (2005) also emphasized that prolonged harvesting interval (i.e. advancing age of maturity) increased dry matter and nutrient variation significantly. The cell wall components

such as cellulose, hemicellulose and lignin, of old plant leaves which are normally at low or browsable height are expected to be higher than new shoots which grows at the tip of branches or non-browsable height of most of the plant. These could be due to the fact that old leaves have been exposed to those factors that stimulate biosynthesis of such components than young ones. Fernandes *et al.* (2013) reported that the concentration level of five different substances varied according to harvesting time, suggesting that the biosynthesis of polar metabolites of *Calendula officinalis* was affected by harvesting time. The accumulation of these cell wall components varies with plant species as they are genetically different. Foster *et al.* (2012) indicated that during accumulation of cell wall components, the level of cell contents such as CP declines during plant growth, and the rate of the decline of CP is greatly influenced by different factors such as species variation and environmental conditions such as temperature, herbivory etc. Photosynthates are quickly converted to structural concentration, leading to a decline in nitrate, protein and soluble carbohydrate content and increased cell wall composition. This postulates that the chemical content of plant leaves will differ with harvesting height, as those at the tip of the tree and their branches will differ with those at the base or lower heights.

As animals browse the leaves, this stimulates plant defense strategy which could be inductive or constitutive, as plants produce and accumulate secondary metabolites, such as tannins as a defense mechanism to protect the plants from mechanical damages from herbivores (Ward & Young, 2002; Skarpe & Hester, 2008). Therefore it is crucial to determine nutritional composition of forage at different plant heights as there are biochemical processes and interaction with their environment which tend to influence a plant's chemical composition. This knowledge will benefit farmers as they will know the potential nutritional value of their feed and the amount of supplementation needed to meet the nutritional requirement of their livestock. Therefore, the

objective of this research was to evaluate the nutritional composition of leaves from *M. capitata, O. africana, C. rudis, C. macrocarpa, R. refracta, Z. mucronata, B. oliedes, G. robusta, P. vessucosus* and *E. rigida* tree species harvested from two different harvesting heights. This chapter verified the alternative hypothesis that plant species and harvesting height can cause variation in the nutritive value of tree leaves.

3.2 Materials and Methods

3.2.1 Study sites, leaf sampling and processing

All leaf samples were collected from Soxhada communal grazing area located at S32°46.907' and E026°51.533' near University of Fort Hare, Alice campus, in the Eastern Cape Province. It is situated in the False Thornveld of the Eastern Cape (Acocks, 1975). The topography of the area is generally flat with a few steep slopes and stoney soil (Ngambu *et al.*, 2013). The vegetation is a mixture of some trees, shrubs, and grass species. The area is predominantly savanna and comprising of subtropical thicket vegetation dominated by deciduous woody shrubs shorter than 1.5 m although the woody layer can reach up to 5 m (Scogings *et al.,* 1996). The area is predominantly used by livestock, comprising of cattle, goats and sheep and a few wildlife species such as kudu. The herbivores spend about 33% of their feeding time browsing at an average height of 1.45 m, though more than half of this time could be spent browsing below the 1.45 m level (Dutoit, 1990). Rainfall in the area ranges from 70 mm to 400 mm per annum and altitude ranges from 500 m to 530 m above sea level. Temperature ranges from -5 °C–18.4 °C in winter and 15 °C–30 °C in summer (Mabidi, 2016).

Leaves were harvested from a total of ten browse tree species namely; *M. capitata, O. africana,C. rudis, C. macrocarpa, R. refracta, Z. mucronata, B. oliedes, G. robusta, P. vessucosus* and *E. rigida* that were identified as important and common browse species used by livestock through household interviews, which were conducted in a preliminary survey. The height was determined using measuring tape, where the height was established by measuring from the ground level to both <1.5 m and >1.5 m and leaves were individually plucked, simulating browsing livestock. Step ladder was used to reach higher leaves.

For each species, five individual trees were marked and leaves were simultaneously collected from two height levels; browsable height (<1.5 m) and non-browsable height (>1.5 m) from each tree thus generating a total of 100 samples. Leave samples from each respective tree species were harvested from each respective height, were thoroughly mixed, and replicated into sampling paper bags, which were labelled in accordance with tree species and harvesting height. They were oven-dried at 60°C to protect heat liable substances such as phenols (Garcia, 2013; Belachew *et al.*, 2013; Mnisi, 2015) until constant weight, milled (2 mm sieve) (Polymix PX-MFC 90 D), and stored pending laboratory analysis. Laboratory analyses (chemical composition, soluble phenols and condensed tannins) of dried and ground samples were then conducted in the Animal Science Laboratory at Molelwane, North-West University, at Mafikeng.

3.2.2 Proximate and mineral analysis

Laboratory dry matter (DM) and OM of leaf samples were determined according to method 973.18 of the (AOAC, 2012). The dry matter (DM) was determined by weighing about 1 g of respective tree samples into pre-weighed crucibles then dried at 60°C until constant weight in an oven. Moisture content was determined as weight lost while DM was determined as change from

initial sample weight. The amount of organic matter in the dried samples was established by burning them to ash at 600°C for about 6 hours in muffle furnace. Organic matter (OM) was calculated as weight lost after ashing and the residue was measured as ash. Neutral and acid detergent fibre (NDF and ADF) and acid detergent lignin (ADL) were determined by the method described by Van Soest *et al.* (1991). Briefly, approximately 0.45 g samples were refluxed with respective NDF and ADF solution for about one hour using ANKOM^{2000} Fibre analyser (ANKOM Technology, New York). Cellulose determined by subtracting ADL results from ADF. For neutral detergent fibre, heat-stable α-amylase was used but not sodium sulphite. The results of both fibre and residual ash content were presented as g/kg DM, inclusive of residual ash.

Nitrogen content was determined for each plant sample using the Kjedahl method number 976.06 (AOAC, 2012) and expressed in g/kg N. The mineral content of forages leaves: calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were determined using the Inductive Coupled Plasma Mass Spectroscopy according to method no. 984.13 (AOAC, 2012).

3.2.3 Extraction and analysis of soluble phenolics

Determination of soluble phenolics was done according to the Folin-Ciocalteau method used by Makkar (2003). Absorbance was read at 725 nm wavelength using a spectrophotometer (T60 UV-Visible, PG Instruments Limited, Beijing, China). A standard curve was developed using tannic acid to predict the concentration of soluble phenolics in leaf samples, which was expressed as tannic acid equivalents (TAE).

3.2.4 Condensed tannin extraction and quantification

The aqueous acetone extract of soluble phenolics from 3.2.3 was transferred into a test tube using a pipette and 3 mL of butanol–HCl reagent was added. The test-tubes were covered and heated at 100°C on a heating block for one hour and allowed to cool to room temperature. The absorbance was determined with spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments Limited, Beijing, China), at a wavelength of 550 nm and reported as absorbance units (AU).per 200 mg sample (Makkar, 2003)

3.3 Statistical analysis

Analysis of variance for chemical composition data was done using the GLM procedures of SAS 9.3 (2010) for a factorial treatment arrangement in a Complete Randomized Design (CRD) was used. The main effects were harvesting height (browsable and non-browsable heights) and tree species. The general linear model was as follows:

$$Yijk = \mu + Si + Hj + (SxH)ij + \varepsilon ijk$$

Where denotes the overall mean, S_i is the effect of tree species, H_j is the effect of harvesting height, $(S \times H)_{ij}$ is interaction effect of species with plant height and is the random error associated with observation *ijk* assumed to be normally and independently distributed. Where significant variation was detected, multiple comparisons of treatment means were carried out using the probability of difference (pdiff) option of the GLM procedure of SAS (2010). For all statistical tests, significance was declared at $P < 0.05$.

3.4 Results

3.4.1 Proximate composition

Table 3.1 presents the DM and cell wall components (NDF, ADF, ADL and cellulose) of leaves harvested from *M. capitata, O. africana, C. rudis, C. macrocarpa, R. refracta, Z. mucronata, B. oliedes, G. robusta, P. vessucosus* and *E. rigida* trees. The DM and cell wall components were affected (P <0.05) by species only. The interaction between browse species × harvesting height had no effect (P >0.05) on DM and cell wall components of browse tree leaves. The results show that the laboratory DM ranged from 912.4 to 960 g/kg with *E. rigida* having the lowest (P <0.05) DM whilst *R. refracta* leaves had the highest ($P < 0.05$) DM.

The NDF content of leaves ranged from 307.1 g/kg DM (*C. rudis*) to 684.4 g/kg DM (*Z. mucronata*). *Ziziphus mucronata* was followed by *G. robusta* and *R. refracta*, which had a different ($P < 0.05$) level of NDF. *Olea africana* had statistically similar ($P > 0.05$) amount of NDF as *P. vessucosus* but higher ($P < 0.05$) than *C. rudis,* which accumulated the lowest ($P < 0.05$) amount of NDF. Leaves from the ten browse plant leaves had different (P <0.05) amounts of ADF. The concentration of ADF in *Maytenus capitata* (350.7 g/kg DM) was the highest ($P < 0.05$) whilst *R. refracta* (185.2 g/kg DM) had the lowest (P <0.05). *Maytenus capitata* was followed by *E. rigida* and *C. macrocarpa*, whose ADF levels were higher ($P < 0.05$) than in leaves of other tree species. Table 3.1 also reveals that ADL content of the leaves ranged from 123.4 (*G. robusta*) to 199.5 g/kg DM (*M. capitata*).

Table 3.1: Dry matter (g/kg) and cell wall (g/kg DM) composition of different browse plants

	Chemical components[1]				
Browse spp	DM	NDF	ADF	ADL	Cellulose
Boscia oleoides	942.7^{b}	429.9^{d}	263.0^{c}	142.8^{d}	120.2^{bc}
Carissa macrocarpa	941.7^{b}	413.1^{d}	331.6^{b}	187.5^{ab}	144.0^{ab}
Coddia rudis	944.4^{b}	307.1^{f}	208.7^{e}	159.6^{c}	49.1^{d}
Ehretia rigida	912.4^{e}	448.7^{dc}	335.4^{b}	180.4^{c}	155. 0^{ab}
Grewia robusta	932.6^{c}	638.3^{b}	255.5^{c}	123.4^{d}	141.6^{b}
Maytenus capitate	946.9^{b}	447.8^{dc}	350.7^{a}	199.5^{ab}	151.2^{ab}
Olea africana	946.4^{b}	371.1^{e}	320.4^{b}	185.6^{ab}	134.8^{b}
Phyllanthus vessucosus	923.3^{d}	347.6^{e}	227.9^{d}	124.5^{d}	103.4^{c}
Rhus refracta	960.0^{a}	587.0^{c}	185.2^{f}	143.7^{d}	52.4^{d}
Ziziphus mucronata	931.6^{dc}	684.4^{a}	254.0^{c}	135.2^{d}	118.9^{cb}
SEM	4.5	14.6	6.5	12.7	12.3

[1]Chemical components: DM = Dry matter; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; ADL = Acid detergent lignin

^{a,b,c,d,e,f,g} In a column, means with common superscripts do not differ (P >0.05)

Ehretia rigida and *C. rudis* had higher ($P < 0.05$) ADL content than *B. oliedes, P. vessucosus, R. refracta, Z. mucronata* and *G. robusta,* which accumulated the least ($P < 0.05$) ADL levels. Cellulose content in the leaves of the ten browse plants is also shown in Table 3.1. It shows that *R. refracta* leaves had the lowest ($P < 0.05$) amount of cellulose (49.1 g/kg DM) while *C. rudis* (155.0 g/kg DM) recorded the highest level.

The results for ash, soluble phenolics and total N are presented in Table 3.2. There was a significant ($P < 0.05$) influence of tree species, harvesting height and tree species × harvesting height interaction on soluble phenolics and total nitrogen concentration of leaves while ash was affected by the interaction only. The amount of ash in leaves harvested at browsable height from the ten tree species ranged from 50.0 g/kg DM (*O. africana*) to 120.6 g/kg DM (*E. rigida*) while the leaves from non-browsable height ranged between 50.9 g/kg DM (*O. africana*) to 118.9 g/kg DM (*E. rigida*). Leaves of *E. rigida* had the highest ($P <0.05$) ash content whilst *O. africana* leaves had the least ash content at both harvesting heights. For leaves harvested at the non-browsable height, *E. rigida* had the highest ash level of 118.9 g/kg DM followed by *M. capitata* (94.2 g/kg DM), *G. robusta* (85.7 g/kg DM), *Z. mucronata* (81.4 g/kg DM) and *C. rudis* (81.4 g/kg DM). *Olea africana* leaves had the lowest ash content (50.9 g/kg DM) at the non-browsable height. The results shows that tree species as well as an interaction between tree species and harvesting height had a significant ($P <0.05$) effects on soluble phenolic content. The results shows that soluble phenolic content for leaves harvested at the browsable height, ranged between 553.2 µg TAEQ /kg DM for *B. oliedes* and 744.1 µg TAEQ /kg DM for *R. refracta,* while at non-browsable height, *Z. mucronata* leaves (560.9 µg TAEQ /kg DM) had the least ($P <0.05$) whilst *M. capitata* leaves (737.6 µg TAEQ /kg DM) had the highest ($P <0.05$) concentration of soluble phenolics.

Table 3.2: Effects of tree species and harvesting height (browsable (< 1.5 m) and non-browsable (> 1.5 m)) on ash (g/kg DM), soluble phenolics (SPh, µg TAEQ /kg DM) and total nitrogen (g/kg N) content of leaves

	Ash		SPh		Total N	
Browse plants	< 1.5 m	> 1.5 m	< 1.5 m	> 1.5 m	< 1.5 m	> 1.5 m
Boscia oleoides	73.4^{E}	72.5^{F}	553.2^{bC}	650.2^{aC}	19.5^{bD}	18.2^{aE}
Carissa macrocarpa	80.4^{D}	77.5^{E}	718.7^{aA}	724.7^{aA}	13.0^{aH}	13.1^{Ha}
Coddia rudis	77.8^{D}	84.5^{DC}	555.4^{bC}	688.7^{aAB}	14.9^{aG}	15.0^{Fb}
Ehretia rigida	120.6^{A}	118.9^{A}	647.3^{aB}	636.0^{aB}	18.3^{aE}	18.7^{aE}
Grewia robusta	85.6^{C}	85.7^{C}	675.2^{aBA}	725.2^{aA}	21.4^{bB}	22.7^{aB}
Maytenus capitate	99.5^{B}	94.2^{B}	741.0^{aA}	737.6^{aA}	13.1^{aH}	13.1^{Ha}
Olea africana	50.0^{G}	50.9^{G}	732.5^{aA}	730.1^{aA}	20.4^{aC}	20.5^{Ca}
Phyllanthus vessucosus	57.1^{F}	54.4^{G}	709.3^{aA}	712.7^{aA}	17.6^{aE}	18.6^{Ec}
Rhus refracta	77.8^{D}	73.4^{F}	744.1^{aA}	717.9^{aA}	17.3^{FEb}	19.6^{aD}
Ziziphus mucronata	79.2^{D}	81.4^{D}	708.4^{Bc}	560.9^{Ac}	25.0^{Ab}	27.0^{aA}
SEM	1.9		27.5		0.4	

^{ABCDEFG} In a column, different uppercase superscripts denote significant (P <0.05) differences between tree species.

^{ab} In a row, common lowercase superscripts denotes non-significant differences (P >0.05) between harvesting heights.

Table 3.2 also shows that interaction between tree species × harvesting height had a significant ($P < 0.05$) influence on total N level of the leaves. Tree species also had influence ($P < 0.05$) on N accumulation whereas harvesting height did not affect N concentration. *Ziziphus mucronata* (27.0 g/kg N) and *G. robusta* (22.7 g/kg N), had the highest total N at non-browsable height. They were then followed by *R. reflecta* (19.6 g/kg N) and *P. vessucosus* (18.6 g/kg N) leaves harvested at the non-browsable height, respectively. *Carissa macrocarpa* (13.0 g/kg N) and *M. capitata* (13.1 g/kg N) leaves had the lowest level of total N at non-browsable height. For leaves harvested from the browsable height, *Z. mucronata* had 25.0 g/kg N of total N, which was the highest ($P < 0.05$) of all ten browse leaves. *Grewia robusta* (21.4 g/kg N) leaves had the second highest ($P < 0.05$) N content. Browse leaves that contained the lowest total N at browsable height were from *C. macrocarpa* and *M. capitata* tree species, which had 13.0 and 13.1 g/kg N content, respectively.

The browse species and harvesting height had a significant ($P > 0.05$) influence on concentration of condensed tannins (CTs) in the leaves, while their interaction did not affect its accumulation. The CTs concentration in these browse leaves ranged from 0.12 $AU_{550\ nm}$/200 mg (*B. oliedes*) to 1.04 $AU_{550\ nm}$/200 mg (*C. macrocarpa*). The harvesting height also had significant influence ($P < 0.05$) on CTs content in the browse leaves, as on average, non-browsable (0.61 $AU_{550\ nm}$/200 mg) was higher than at browsable height (0.55 $AU_{550\ nm}$/200 mg). Leaves of *C. rudis, G. robusta* and *Z. mucronata* harvested at non-browsable height had higher condensed tannin content than those harvested at browsable height.

3.4.2 Macro and micro-minerals

The results on concentrations of macro-minerals of leaves from the ten browse species are presented on Table 3.3. Only browse tree species had effect ($P < 0.05$) on macro-minerals as plant height and their interaction had no significant influence on macro-mineral content of leaves. Phosphorus concentration was significantly different ($P < 0.05$) on browse species while harvesting height did not impact ($P > 0.05$) P concentration in browse leaves. The results show that P content of leaves ranged from 0.6 (*B. oliedes*) to 2.9 mg/kg DM (*E. rigida* and *Z. mucronata*). They were followed by *C. macrocarpa*, which was higher than *O. africana, P. vessucosus* and *G. robusta*, which differed ($P < 0.05$). *Coddia rudis* and *R. refracta* had statistically similar P levels, which were higher than in *M. capitata* leaves while the lowest P levels were observed in *B. oliede* leaves.

The K content of browse leaves ranged from 7.6 (*B. oledies*) to 32.0 mg/kg DM (*E. rigida*). The results show that *E. rigida*, had the highest K, and was followed by *R. refracta* and *Z. mucronata* which had similar ($P > 0.05$) concentration of K and higher ($P < 0.05$) of other tree species. The next rank was occupied by *C. rudis, P. vessucosus* and *M. capitata* whose K levels were also similar ($P > 0.05$). The rest of the tree species ranked as *G. robusta* > *O. africana* > *C. macrocarpa* > *B. oliede* in the descending order of K levels which were different ($P < 0.05$).

Table 3.3: Macro-mineral content (g/100 g DM) of leaves from different browse plants

Species	Phosphorus	Potassium	Calcium	Magnesium
Boscia oleoides	0.6^{h}	7.6^{h}	15.1^{d}	5.6^{b}
Carissa macrocarpa	2.8^{b}	13.2^{g}	24.4^{b}	3.8^{c}
Coddia rudis	1.5^{f}	17.8^{d}	16.9^{c}	3.4^{c}
Ehretia rigida	2.9^{a}	32.0^{a}	26.3^{a}	7.2^{a}
Grewia robusta	1.7^{e}	16.1^{e}	25.9^{a}	3.3^{c}
Maytenus capitata	1.2^{g}	17.3^{de}	23.6^{b}	7.8^{a}
Olea Africana	2.5^{c}	14.7^{f}	11.3^{e}	1.6^{d}
Phyllanthus vessucosus	2.1^{d}	17.0^{de}	9.9^{f}	1.4^{e}
Rhus reflecta	1.4^{f}	22.0^{c}	8.7^{f}	2.2^{d}
Ziziphus mucronata	2.9^{a}	24.3^{b}	16.1^{cd}	1.9^{d}
SEM	0.05	0.5	0.6	0.3

^{a,b,c,d,e,f,g} In a column, means with common superscripts do not differ (P >0.05).

The results presented in Table 3.3 reveal that Ca concentration in browse leaves was also not influenced (P >0.05) by interaction between tree species and harvesting height. It shows that Ca level was significantly (P <0.05) affected by tree species and it ranged from 8.7 to 24.3 mg/kg DM with *E. rigida* and *G. robusta* leaves containing the highest levels. *Carissa macrocarpa* and *M. capitata* had similar (P >0.05) Ca concentration, which were the second highest. The lowest

levels of Ca were observed in leaves harvested from *B. oliede*. *O. africana*, *P. vessucosus* and *R.refracta* tree species. There was no significant effect due to interaction on Magnesium content from ten browse leaves as presented in Table 3.3. It shows that only browse species had effect ($P < 0.05$) on Mg concentration of browse leaves, harvesting height did not have an impact. Its concentration ranged from 1.4 (*P. vessucosus*) to 7.8 mg/kg DM (*M. capitata*).

The results on the influence of tree species, harvesting height and their interaction on micro-mineral composition of tree leaves are presented in Table 3.4. It shows that only browse plant species affected concentrations of different micro-minerals. The Fe content of trees leaves ranged from 78.7 (*P. vessucosus*) to 189.4 g/100 g DM (*C. rudis*). Leaves from *Z. mucronata* and *C. rudis* had the highest ($P < 0.05$) Fe concentration while *M. capitata*, *R. refracta* and *C. macrocarpa* leaves had the second highest Fe concentration, which were higher ($P < 0.05$) than those of *B. oliede* and *O. africana*. Leaves from *E. rigida, P. vessucosus* and *G. robusta* had similar Fe levels, which were the lowest of the ten tree species.

The copper content in the leaves of browse trees is also presented in the Table 3.4. It reveals that *C. macrocarpa*, *O. africana*, *Z. mucronata, E. rigida, P. vessucosus* and *R. refracta* contained different concentrations of Cu. Leaves from *G. robusta* and *M. capitata* had similar Cu level, which was higher ($P < 0.05$) than leaves from *B. oliedes*. The results also show that *B. oliedes* leaves had the highest ($P < 0.05$) level of Zn (96.9 g/100 g DM) followed by *G. robusta,* which had 36.3 g/100 g DM of Zn. *Ziziphus mucronata, R. refracta* and *C. rudis* had similar ($P > 0.05$) levels of Zn, which was the lowest ($P < 0.05$). The concentration of manganese ranged from 22.9 to 206.7 g/100 g DM, with *P. vessucosus* leaves containing the highest ($P < 0.05$) while *O. africana* had the least ($P < 0.05$) Mn content (22.9 g/100 g DM).

Table 3.4: Micro minerals content (g/100 g DM) of different browse leaves

Browse trees	Iron	Copper	Zinc	Manganese
Boscia oleoides	95.3^{c}	2.2^{f}	96.9^{a}	171.0^{b}
Carissa macrocarpa	110.8^{bc}	10.4^{a}	30.6^{bc}	132.1^{c}
Coddia rudis	189.4^{a}	6.1^{d}	11.6^{ed}	69.7^{d}
Ehretia rigida	85.1^{dc}	7.1^{c}	25.7^{cd}	78.0^{dc}
Grewia robusta	79.5^{dc}	4.3^{e}	36.3^{b}	45.9^{e}
Maytenus capitate	123.2^{b}	4.3^{e}	25.7^{cd}	49.0^{de}
Olea africana	93.0^{c}	9.4^{b}	19.8^{d}	22.5^{f}
Phyllanthus vessucosus	78.7^{dc}	6.9^{c}	28.5^{bc}	206.7^{a}
Rhus reflecta	126.2^{b}	6.9^{c}	11.6^{ed}	64.6^{de}
Ziziphus mucronata	173.4^{a}	7.4^{c}	15.7^{ed}	71.7^{d}
SEM	12.5	0.3	4.2	11.4

^{a,b,c,d,e,f,g} In a column, means with common superscripts do not differ (P <0.05)

SEM: Standard error of the mean

3.5 Discussion

3.5.1 Fibre components

The only source of significant variation of fibre fractions was due to tree species. The interaction between tree species and harvesting height did not affect fibre (NDF, ADF, ADL and cellulose) accumulation. The difference in fibre level between plant species could be due to their genetic composition. Since the plants are growing under the same environment and soil, the sole source of varying rate of fibre accumulation and lignification would be their individual response to environment (Van Soest, 1982). The response to environment is mainly influenced by genetic makeup. Statistically insignificant variation due to harvesting height could be due to different factors such as growth pattern or canopy of the tree. Some trees are bushy and spread, this could mean growing new shoots within browsable and non-browsable height hence having leaves of similar physiological age and maturity.

The NDF content of browse leaves from both browsable and non-browsable height was above minimum required for proper rumen function, motility, maintenance of normal and rumen pH (Grant, 1997). The concentration of fibre in the leaves of these ten browse species was within the range (24–61%) reported by other authors and generally for forages used in ruminant feeding (Meissner *et al.*, 1991). The NDF content of leaves is in line with those reported by Fall (1993), who found that NDF of browse leaves ranged between 31 to 57% while Njidda *et al.* (2010a); Njidda *et al.* (2010b) also reported a range of 24-61%. Most of the leaves in the current study had NDF content that was above 35% suggesting that they may have lower digestibility compared to those with lower NDF content. *Coddia rudis* leaves are likely to be the most digestible as they had less than 35% of NDF and their intake is expected to be higher than leaves of the other tree species, under the same conditions. Grant (1997) and Van Soest (1982) indicated that NDF is a

vital source of energy for ruminants. The results of the present study show that *Z. mucronata* had the highest NDF content (684.4 g/kg DM), which is likely to limit feed intake as Njidda (2014) noticed that NDF and ADL are some of the factors that tend to depress dry matter intake and digestibility.

The ADF results were within the range reported by Fall (1993), who found that ADF in tree leaves ranged from 19 to 43 %. Hassen *et al.* (2009) revealed that the ADF of *Z. mucronata* leaves harvested in different seasons of the year ranged between 175 – 312 g/kg DM in agreement with the findings in the current study where ADF content of *Z. mucronata* leaves was 254.0 g/kg DM. In addition, Njidda & Olatunji (2012) reported ADF values from 205.9 to 228.3 g/kg DM for leaves from *Ziziphus* species, a finding also corroborated by the present study. In general, the NDF and ADF are within the range (24-61% and 17-61%, respectively) and are within the range of documented by other researches. Lignin is a part of the cell wall, which is deposited and accumulates as the cell wall-thickening process (Boudet, 1998). It is indigestible and when its concentration is high, it tends to decrease DM digestibility but increase the outflow of small particles from the rumen (Sekineh *et al.*, 1994). The lignin content varies according to species, age and plant parts. The ADL content of leaves in this study ranged from 123.4 to 199.5 g/kg DM and fell within the range reported by other authors. However, Njidda (2010) reported ADL content of leaves from different arid and semi-arid browse trees ranged between 49.0 g/kg to 127.0 g/kg DM. Njidda & Olatunji (2012) also reported that leaves from different *Ziziphus* species show that ADL content ranged from 108.6 to 119.3 g/kg DM, which is lower than the levels observed in the current research (135.16 g/kg DM). On the other hand, Al shafei & Nour (2016) reported ADL values of up to 314.0 g/kg DM in tree leaves. Rittner & Reed (1992) reported a comparable average for lignin contents of browse species across different ecological

zones as follows: 117 g/kg DM in the Sahelian zone 105.0 g/kg DM in the sub-humid zone and 93.0 g/kg DM in the humid zone. The disparity in the chemical composition of browse forages is caused by different factors, which include soil type (location), the plant part (leaf, stem or pod), age of leaf and season (Rittner & Reed, 1992). Van Soest (1982) indicated that a variety of tree species and climate of different environments play a crucial role on the quality of plants, as plants growing in high temperature tend to accumulate high lignin which decreases feed quality. The variation in chemical composition of tree leaves of this study and other previous studies could be due to the above-mentioned factors. Ash content of leaves from various browse plants was within the range (83.9–150.0 g/kg DM) reported by Al shafei & Nour (2016).

3.5.2 Total nitrogen

The moderate to high levels of N in browse leaves is well-documented and is one of the main distinctive characteristic of browse products compared to most grasses. Researchers such as Al shafei & Nour (2016) indicated that browse trees play a vital role as they are a major source of protein-containing feed for livestock in sub-Saharan Africa, which is susceptible to droughts. Knowledge and understanding of N content in these browse plants are important to farming communities hence research on their chemical composition. The results reveal that an interaction between plant species and harvesting height had a significant influence on N content of browse leaves. Tree species also had influence on N accumulation.

All the plant species in the present study have their N level within the acceptable range (110-140 g/kg N) for ruminant diets. They can be used as N supplements especially during dry periods, in the tropics, where low quality roughage usually has lower N concentrations (4.8–8 g/kg N) (Leng, 1990) and they can help to meet the minimum CP requirement of 80 g CP/kg DM needed for

optimal rumen microbial function. So it is important to determine the amount of protein that is rumen digestible for accurate advice on feed formulation. Nitrogen content of browse leaves in the current study ranged from moderate to high with *C. macrocarpa* accumulating the least with 13.0 g/kg N while *Z. mucronata* had the highest amount (27.0 g/kg N). These results are comparable to those reported by Rubanza *et al.* (2003) who found N to range from 16.0–35.2 g/kg N in browse plants. The N content of *Z. mucronata* in the current study was higher than that reported by Njidda & Olatunji (2012) who found that *Z. mucronata* leaves had 153.65 g/kg N while Hassen *et al.* (2009) reported a range of 19.7–41.9 g/kg N across different seasons. On the other hand, Mnisi & Mlambo (2016) reported the highest N content in leaves of *Z. mucronata* as 28.4 g/kg N. The reports by different authors show that N obtained in the current study was within the range of other findings. The conclusion by Al shafei & Nour (2016) emphasized that browse plant leaves contain moderate to high levels of N and they are a crucial source of protein for livestock. The variation in N concentration between species can be due to inherent characteristics of each species related to the ability to extract and accumulate nutrients from soil (Rittner & Reed, 1992; Ogunbosoye *et al.*, 2015).

Harvesting height did not affect N concentration, which was contrary to the reports by other researchers as well as expectations of the current study. Van Soest, 1982; Rittner & Reed (1992), Buxton & Redfearn (1997); Njidda *et al.* (2012) observed that age of the plant leaf is one of the factors that can cause variation on the nutritional value of the leaf. It is also important to note that different plants have varying growth patterns. Some browse plants are bushy and spread hence their new nutritious shoots grow within 1.5 m, while others grow new shoots at the tips of their canopy. Plants with inductive mechanism can also be stimulated by defoliation at browsable height to grow new shoots which are nutritious, and this could contribute to non-

significant variation in total N at different heights.

3.5.3 Phenolics

It is envisaged that a plant would channel its defense substances to young, nutritious plant parts as they are the most susceptible to herbivory, but the exposure and accessibility of leaves by herbivores are other factors that have a crucial effect on distribution of phenolics. The results shows that tree species and their interaction with harvesting height had an influence on soluble phenolic content. There was no significant variation on concentration of phenolics between the two heights. These browse trees are genetically different and they are bound to vary on the biosynthesis and response mechanism to their environment (Ibrahim *et al.*, 1988). Browse plants were growing under the same communal area and exposed to similar environmental factors but their phenolic and tannin content varied, respectively, which could indicate that their genetic material could be the major cause of variation.

Non-significant impact of harvesting height on phenolic was observed and could have been caused by varying plant's growth patterns as some grow tall while others are bushy since these plants channelled phenols to young, nutritious and new shoots as well as the leaves that are exposed and accessible to herbivores (Ward & Young, 2002). This could also mean that inductive mechanisms of plants to produce phenolics on new shoots were activated while leaves were still within browsable height and they were still produced on new shoots growing at non- browsable height. Plants that had leaves with the highest phenolic content at browsable height include *R. refracta, M. capitata, O. africana, P. vessucosus, C. macrocarpa* and *Z. mucronata*. Some of them such as *B. oliedes* are bushy such that new, nutritious leaves would still be growing below and above 1.5 m. The results suggest that plants could be channelling most phenols and tannins to the new, nutritious leaves that are exposed to herbivores or predators as a defense

mechanism to deter browsers. The objective being to protect the newly developing nutritious plant parts, which are mostly selected by the animals and the even mature ones that are still at risk.

Ibrahim *et al.* (1988) indicated that there are various factors that could induce and affect accumulation of secondary plant metabolites. These factors include genetics, abiotic factors, and biotic factors. It is crucial to understand that phenolic concentration in plants is not only affected by herbivory. This could also explain the non-significant effect of harvesting height on phenolic content of leaves as the biotic and abiotic stress factors can also induce their biosynthesis. The leaves of *B. oliedes* and *C. rudis* had a higher phenolic content at non-browsable height than at browsable height. This could suggest that these trees have inductive mechanisms that were activated to protect new shoots growing at the tip of tree. On the other hand *Z. mucronata* leaves had the highest phenolic content at browsable height, which could be due to the new leaves growing within the reach of browsers so the plant concentrated more phenolics as a mode of protection from herbivores (Gowda, 1997). This also reveals that the inductive mechanism for biosynthesis of phenolics is present in some tree species but not in other trees.

The browse species and harvesting height had a significant difference and influence on the concentration of condensed tannins (CT) in the leaves. The CTs concentration in these browse leaves ranged from 0.12 $AU_{550\ nm/200\ mg}$ (*B. oliedes*) to 1.04 $AU_{550\ nm/200\ mg}$ (*C. macrocarpa*). The difference in content of CTs amongst plants could be due to their genetic variations which has varying response mechanisms to environmental stimulae (Petrussa *et al.,* 2013). Donnelly (1959) concluded that the harvesting height was not associated with tannin content. This conclusion is contrary to observation by Petrussa *et al.* (2013), who indicated that flavonoids, such as condensed tannins, are widely distributed according to the plant species, organ, developmental stage and

growth conditions. The results of the current study agrees with Petrussa *et al.* (2013), as they showed that on average, the leaves harvested from non-browsable height (0.61 $AU_{550\ nm}$/200 mg) were higher than at browsable height (0.55 $AU_{550\ nm}$/200 mg). This is contrary to a report by Berard *et al.* (2011), who observed that condensed tannin concentration in forage legume species they investigated was higher in the mature samples than the vegetative samples, which could also be due to inductive mechanism in those plants. The concentration of CTs in *G. robusta* (0.8), *C. rudis* (0.5) and *Z. mucronata* (0.7) leaves harvested from non-browsable height was higher than those harvested at browsable height 0.6, 0.3 and 0.6 $AU_{550\ nm}$/200 mg while there was no significant difference in other species. This could suggest that these browse trees had their new, nutritious shoots growing at the tips of the trees. Since they were browsed (while they were still under browsable height), they distribute CTs, which are astringent and bitter (Ashok & Upadhyaya, 2012), to new shoots to protect and scare browsers. The results of this study show that some plants had high levels of tannins at browsable height while others at non-browsable height. This could suggest that some plants have inductive mechanism that enables them to respond to harsh environmental conditions by producing tannins as a defense mechanism.

Plants that had the lowest condensed tannin levels (*B. oliede, O. africana*, *E. rigida* and *P. vessucosus*) can safely be utilized as protein supplements as there will be low risk of toxicity to some rumen microbes, and the animal would also benefit from rumen degradable and bypass protein provided by protein-tannin complexes (Reed *et al.*, 1990). McMahon *et al.* (2000) stated that is also vital to note that recent evidence suggests that tannin concentration alone cannot be used to predict nutritional effect but the type of tannin and molecular weight. Disparities in CT chemistry changes protein-binding capacities among polymers from different plant species and developmental stages (McMahon *et al.*, 2000). Indeed, Njidda (2014) noted that condensed

tannins reduce digestion of fibre by complexing with lignocelluloses, preventing attachment of microbes that degrade the fibre. Thus low levels of CTs in plants such as *B. oliedes, O. africana* and *E. rigida* will also allow fermentation of fibre for production of energy and degradation of protein to support rumen microbes. Reed *et al.* (1990), Aerts *et al.* (1999) and Fadiyimu *et al.* (2011) noted that at moderate concentrations (<50 mg/g DM), condensed tannins (CTs) can reduce the risk of bloat, increase the uptake of essential amino acids and proteins, improve animal productivity and effectively control gastrointestinal parasites.

3.5.4 Macro- and micro-minerals

Forage plants play a crucial role in the provision of minerals to animals, as they are able to maintain a higher nutritional status even during dry season. Aganga & Mesho (2008) emphasized that minimum mineral intakes must be sufficient to ensure the long-term maintenance of the mineral reserves of the body tissues and the amounts of those minerals in the edible products of the animal. Mineral requirements are largely dependent on the level of productivity, as the productivity increases the amount required also increases. So it is vital to investigate the mineral content of forage as it will inform the supplementation required during the dry seasons.

The interaction between browse species and harvesting height did not influence concentration of all macro minerals in browse leaves. The concentration of different macro-mineral was significantly different between tree species. This difference could have been due to differing genetic material since all these trees grew in the same area, under similar environmental condition. Ibrahim *et al.* (1988); Rittner & Reed (1992) and Ogunbosoye *et al.* (2015) indicated that the ability of the plant to extract nutrients from the soil is different and affected by nutrient status and soil pH. The results show that the macro mineral concentration of browse leaves in our current

study were lower than for browse analysed by Aganga & Mesho (2008) but higher than those reported by (Njidda 2010). This could be an indication that genetics (genus, species and variety) and different growth environment of browse plants affects their chemical composition.

Results show that K, which functions as a cation of the cells especially in nerve and muscle excitability as well as carbohydrate metabolism, ranged between 7.6 to 32.0 g/100 g DM. McDonald *et al.* (2002) indicated that K is normally above 25 g/kg DM in plants. The P content of leaves in the present study ranged between 0.6 and 2.9 g/100 g while Aganga & Mesho (2008) reported 3.2 to 4.9 g/100 g DM of P in 22 browse plants they analysed, including *Z. mucronata* (1.88%) and *E. rigida* (1.89%) in Botswana. Calcium, which is the most abundant mineral in the animal body as a vital constituent of the skeleton and teeth, ranged from 8.7 to 24.3 mg/kg DM in the current study whilst Aganga & Mesho (2008) reported that Ca content ranged from 13.8 to 55.1 mg/kg DM in browse tree leaves in Botswana. On the other hand, Njidda (2014) found that Ca levels ranged from 0.75 to 1.95 mg/kg DM in the browse plant leaves they analysed in Nigeria. McDonald *et al.* (2002) indicated that it is vital to note that extraction mechanism of minerals is not yet established but there are various factors that affect absorption of minerals. Calcium absorption is regulated by 1,25-dehydroxycholecalciferol and it is favoured by low alimentary pH, presence of oxalate and phytates and excess amount of P. Magnesium concentration ranged from 1.4 to 7.8 mg/kg DM higher than results (0.15 to 1.09 % DM) by Njidda (2010) while the results (2.4 to 6.2 mg/kg DM) reported by Rubanza *et al.* (2006) were within the range of those of current study. On the other hand, Aganga & Mesho (2008) in Botswana, reported that *E. rigida* and *Z. mucronata* contained Ca levels of 1.89 and 1.88 % while Mg was 0.61 and 0.32%, respectively. On the leaves of six browse plants analyzed by Rubanza *et al.* (2006) Ca value ranged from 13.8 - 55.1 (Ca) while that of P were from 3.2 to 4.9

(P). However, in the current study *E. rigida* and *Z. mucronata* had Ca level of 2.6 and 16.1 mg/kg DM while Mg content was 0.61 and 0.32 mg/kg DM, respectively. McDowell *et al.* (2000) indicated that growing lambs and lactating ewes require 0.9–5.3 and 1.2–3.7 % DM of dietary Ca respectively, while goats need 0.21 – 0.52%, and the results of the present study are within the range of these standards. Ogunbosoye *et al.* (2015) indicated that the level of minerals in forage depends on four major factors, which include genetics (genus, species and variety), location (soil on which forage grew in), climate, and age of plant. The interaction between browse species and harvesting height did not influence concentration of all macro minerals in browse leaves. The concentration of different macro-mineral was significantly different between tree species. This different could have been due to differing genetic material since all these trees grew in the same area, under similar environmental condition.

Micro minerals of these ten browse leaves were also determined, as they are also vital in animal health and production although the body needs them in minute quantities. Iron concentration was high in leaves of *C. rudis* and *Z. mucronata.* All the browse samples analysed had sufficient Fe level to meet requirements for dairy and beef cattle (50 mg/kg DM) though they are below level reported by Ogunbosoye *et al.* (2015), though it is crucial to note that not all nutrients in forage are bioavailable to the animal as explained by McDonald *et al.* (2002). Copper plays a vital role in haemoglobin formation and is a vital component in a number of enzymes in plants. The level of Cu in tree leaves investigated in the current study were in same range as those reported by Ogunbosoye *et al.* (2015) who indicated that the browse species evaluated in their study were deficient in Cu and thus would not meet the daily requirements (18 mg/kg DM) of range goats. Barde *et al.* (2014) reported that Cu ranged from 4.70 to 5.90 (mg/kg DM) while Mn was 19.83 to 21.60 (mg/kg DM) in the leaves of *Sweitenia mahogany*, *Mangifera indica*, *Gmelina arborea*,

Vitex doniana and *Ficus thoningii*. In the present study, *Z. mucronata* had the highest level of zinc of 15.7 g/100 g DM, falling short of critical dietary level (30 mg/kg DM) Ogunbosoye *et al.* (2015).

3.6 Conclusions

Forage leaves from browse plants plays a crucial role in the provision of non-conventional feed, which is more nutritious than grass. Browse plants harvested at browsable height that contained moderate to high N include *Ehretia rigida*, *Boscia oleoides*, *Olea africana*, *Grewia robusta* and *Ziziphus mucronata*. They contain moderate to high level of protein, which can be used to supplement for animals feeding on poor quality roughage grass, especially during the dry season. The different leaves contained high concentration of some of macro and micro-minerals. It is advisable to supplement minerals such as zinc and copper that were below nutritional requirement of animals.

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4 CHAPTER FOUR: BUFFER SOLUBLE NITROGEN AND SIMULATED RUMINAL FERMENTATION OF BROWSE LEAVES HARVESTED AT DIFFERENT HEIGHTS

Abstract

The purpose of this study was to determine the effects of browse species and harvesting height on buffer nitrogen solubility index (NSI), *in vitro* ruminal dry matter degradability (IVDMD), *in vitro* ruminal N degradability (IVND), dry matter and nitrogen degradable parameters of browse leaves from ten tree species. Leaves were harvested from *Maytenus capitata, Olea africana, Coddia rudis, Carissa macrocarpa, Rhus refracta, Ziziphus mucronata, Boscia oliedes, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida* trees growing in Soxhada communal grazing area of Eastern Cape Province, South Africa. The leaves were harvested at browsable (<1.5 m) and non-browsable (>1.5 m) heights. The interaction between browse species × harvesting height had a significant impact on the solubility index of browse species. The NSI of leaves harvested from browsable height ranged from 14.2% (*E.rigida*) to 54.4% (*B. oleoides*) while it was between 54.64% (*B. oleoides)* and 13.9% (*E. rigida*) for leaves harvested from the non-browsable height. The study revealed that plant species and harvesting height and their interaction had influence (P <0.05) on IVDMD and IVND at 12, 24 and 36 hours of incubation. The 12h IVDMD ranged from 178.3 g/kg DM (*E. rigida* leaves harvested from non-browsable height) to 431.33 g/kg DM (*C. rudis* leaves harvested from browsable height). For *Coddia rudis*, leaves harvested from browsable height had higher IVDMD than leaves harvested from non- browsable height. At non-browsable height, *B. oleoides* (291.2 g/kg DM) had the highest (P <0.05) immediately degradable dry matter 'a' fractions while at browsable height *R. refracta* (292.4 g/kg DM) had the highest 'a'. Leaves of *E. rigida* harvested from both non-browsable (455.8 g/kg DM) and

browsable (729.5 g/kg DM) height had the highest degradable part of insoluble 'b' fraction of dry matter. *Boscia oleoides* leaves harvested from non-browsable (0.131) and browsable height (0.037) had the highest ($P < 0.05$) disappearance rate of 'b' fraction. The IVDMD of browse leaves was low for both plant heights, which could be due to high fibre and moderate to high levels of tannins in the leaves observed in the previous Chapter 3. The leaves of *B. oleoides* harvested from both heights had the highest ($P < 0.05$) IVND at 12, 24 and 36 hours of incubation, it also had the highest immediately degradable 'a' fraction of N in leaves harvested from both heights. *Boscia oleoides* was followed by leaves harvested from *P. vessucosus, M. capitata*, *G. robusta* and *C. rudis* from browsable height suggesting that it can be used as N source for ruminal microbes during dry season for ruminants browsing or supplemented with browse foilages when the quality of pasture is poor and fibrous. This study showed that NSI is a poor predictor of *in vitro* ruminal N degradability for all leaves of browse plants except for *P. vessucosus*.

Key words: browse species, plant height, nitrogen solubility index, dry matter digestibility, nitrogen degradability

4.1 Introduction

Browse plants play a crucial role in bridging the gap of seasonal availability of forage, which is common in arid and semi arid regions. Tefera *et al.* (2008) noted that these plants remain green and have higher protein content than grasses during the dry periods that last for about eight months. Though there is scanty research on some aspects of browse plant biology and physiology with regard to livestock nutrition such as secondary metabolic compounds, Achakzai *et al.* (2009) noted that browse plants are able to retain most of their nutrients, including crude protein, which is one of the most important nutrients in ruminant nutrition as it is a source of nitrogen to rumen microbes. The quality of protein is important in ruminant diet and Mnisi & Mlambo (2016) emphasized that the rumen protein degradability is an important indicator of quality of protein. Since forage intake is a function of the rate of forage degradation by ruminal microbes, the rumen capacity, the forage digestibility and passage rate through the gut (McDonald *et al.* 2002), it is vital to determine forage degradability and availability of the resulting products from such processes to the animal. Edwards *et al.* (2012) emphasized that *in vitro* ruminal protein degradation characteristics of browse species can represent an accurate measure of the quality of protein for ruminant animals. Determination of rumen degradable protein is important as it helps to reveal amount of rumen degradable and undegradable protein, as the diet has to provide at least 80 g/kg DM to meet rumen microbes requirements (Aderinboye *et al.*, 2016).

As a plant grows, it is exposed to various factors that alter its chemical composition, resulting in biosynthesis of various plant secondary metabolites. Some of these metabolites form complexes with other plant components and thus modifying digestibility and bioavailability of some nutrients. Beever & Mould (2000) observed that the nutritive value of browse plant leave mainly vary due to differing genetic make up of plant species, edaphic factors, harvesting stage and height as

well as processing of leaves. It is common knowledge that harsh conditions such as high temperature, drought, and herbivory induce stress in plants (Said-Al *et al.*, 2009; Ramakrishna & Ravishankar, 2011; Krasensky, 2012; Hasanuzzaman *et al.*, 2013). In response, plant biosynthesize substances such as secondary plant metabolites like phenolics and accelerate deposition of cell wall components such as fibre and lignin as a defense mechanism. Unfortunately, these responses tend to affect digestibility and availability of some nutrients to the animals browsing on these forage trees. Condensed tannins, which are secondary plant metabolites, are known for these characteristics of reduced ruminal degradability and availability of nutrients. Douglas *et al.* (1995) reported that condensed tannins may form rumen- stable complexes with feed proteins, thereby protecting part of the protein from ruminal degradation, which may improve the protein supply. On the other hand high concentration of condensed tannins may reduce N availability to rumen microbes affecting their population and activity.

One of the methods that can be used as an alternative to *in sacco* and *in vivo* evaluation of protein is buffer solubility. Hedqvist (2004) indicated that increasing knowledge of ruminal degradation of buffer soluble proteins for browse species can be vital for animal productivity. The results can be correlated with those of *in vitro* protein degradability for verification. Aufrere *et al.* (1991) reported that the correlation between buffer solubility of a protein and rumen degradability has not been fully universally demonstrated, but has been shown to depend, largely, on type of feed and type of protein, among other factors. Stern & Satter (1984) and Van Soest (1994) highlighted that N solubility has poor predictive ability to measure bypass protein amongst feeds and its classification of proteins by their solubility is insufficient as it ignores the presence of true proteins and NPN (peptides, amino acids, ammonia and other end-products from fermentation). Therefore the objective of this study was to determine the effects of browse species

and harvesting height on NSI, IVDMD, and IVND as well as correlation between IVND and SI.

4.2 Materials and methods

4.2.1 Study sites and leaf sampling and processing

The sample site, procedure and processing are similar to the one elaborated in Chapter 3. Determination of nitrogen buffer solubility, *in vitro* ruminal degradability of dried and ground samples were conducted in the Animal Science Laboratory at Molelwane, North-West University.

4.2.2 Nitrogen buffer solubility solution

Buffer soluble N of browse leaves was analysed using a procedure by Licitra *et al.* (1999), which was modified by Cudjoe & Mlambo (2014); whereby ANKOM F57 filter bags (ANKOM Technology, New York) were used for the filtration process. The filter bags were weighed and labelled with a solvent-resistant marker and filled with 0.45–0.5 g of leaf samples and sealed with an impulse heat sealer. The samples were then incubated in ANKOM Daisy^{II} incubator jars which were filled with 1500 mL of borate-phosphate and 10% sodium azide buffer solution. This mixture was pre warmed and incubated for 3 hours with agitation in an incubation chamber set at 39°C. After 3 hours the bags were removed, washed with cold water, air dried and then oven-dried at 105°C for 12 hours. The bags were then cooled in a desiccant pouch then weighed.

Nitrogen in the residue was analysed according to the standard macro-Kjeldahl procedure (No. 984.13: AOAC, 2012) and measured as insoluble N content of the samples. The difference between total N and insoluble N content of leaf sample was referred to as soluble N content. Buffer nitrogen solubility index was calculated as a ratio between soluble N and total N content.

4.2.3 Ruminal degradation

Leaf samples were incubated in Daisy^{II} incubator chamber that has a thermostat to maintain a 39°C temperature with four rotating jars, in accordance to ANKOM Technology Method 3 for *in vitro* true digestibility (ANKOM Technology Corp., Fairport, NY). Samples were milled (2 mm), weighed into ANKOM F57 bags (0.45–0.5 g), which were subsequently heat sealed prior to incubation in rotating jars filled with warm (39°C) buffer solution. Buffer solution was a mixture of two buffers at ratio 1:5 and was prepared according to ANKOM Technology Method 3. Each of the four digesting jars was filled with about 1600 mL of combined buffer solution and warmed at 39°C.

Collection of rumen fluid for inoculation was done in the morning before feeding. The rumen fluid was collected from a rumen cannulated Bonsmara cow, which weighed about 550 kg live weight and had been fed blue buffalo grass mixed with lucerne with *ad libitum* access to clean water in a free stall where it was kept. Rumen liqour was collected into two pre-warmed thermos flasks purged with carbon dioxide gas and immediately taken to the laboratory for processing and inoculation. Processing involved blending and straining of rumen fluid through two layers of warm muslin cloths. Rumen fluid was blended so that the microbes that may be attached to fibrous particles maybe dislodged. During the processing, rumen fluid was continuously flush with carbon dioxide gas to maintain an anaerobic environment. The four Daisy^{II} jars, already containing 1600 mL of buffer solution and samples, were purged with CO_2 before being inoculated with 400 mL of strained rumen fluid after which they were tightly closed and incubated in Daisy^{II} incubator at 39 °C. Samples were withdrawn at 0, 2,4,6,12, 24, 36, 48 and 72 hours after incubation, but only 12, 24 and 36 withdrawal points will be presented since they represent points when degradability is actively at pick (Salem, 2006; Isah *et al,* 2012; Mnisi, 2015). Upon

withdrawal of bags at respective periods, they were washed in ANKOM^{2000} Fibre Analyser with running cold water for 20 minutes then air, then oven dried at 105°C for 12 hours, cooled in dessicator, then weighed with analytical balance. The residues were then used to determine N using macro-Kjedahl procedure (AOAC, 2012 Method No. 984.13). The loss from total N upon incubation was referred to as nitrogen degradability. *In vitro* ruminal DM and N degradability for each withdrawal point was determined using the following formula:

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

Whereby: W1 = empty bag weight, W2 = weight of sample, W3 = bag + residue weight after daisy incubation and C1 = Blank bag correction factor (W3 + W1)

Degradation parameter for both dry matter (DMD) and nitrogen (N) were estimated by fitting degradation data, using using Datafit 9 (Oakdale Engineering) to equation by Orskov & McDonald (1979):

$$Y = a + b(1 - e^{-c(t-lt)})$$

Where;

a = soluble fraction, b = insoluble but potentially degradable fraction, c = degradation rate constant of fraction b, t = incubation time (12, 24 and 36 hours), l = lag time (hours),

e = base for natural logarithm.

Effective degradabilities for dry matter (EDDM) and nitrogen (EDN) were estimated using Orskov and McDonald (1979) parameters as follows;

$$ED = a + (bc / k + c)$$

where:

a = immediately soluble fraction, b = insoluble but potentially degradable fraction, c = degradation rate constant of the b fraction, k = rumen outflow rate (assumed to be 0.05/h) due to complex formed between tannins, protein and carbohydrates, which tend to slow the rate of degradation.

4.3 Statistical analysis

4.3.1 Two-way analysis of variance

Buffer N solubility and *in vitro* ruminal degradability of DM and N in *Maytenus capitata, Olea africana, Coddia rudis, Carissa macroparpa, Rhus refracta, Ziziphus mucronata, Boscia oleoides, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida* leaves from different heights was analysed using the general linear models (GLM) procedure of SAS 9.3 (2010) for a 10 x 2 factorial treatment arrangement in a completely randomized experimental design. Main variables were tree species and harvesting height (<1.5 m and >1.5 m). For degradability data, statistical analysis was done separately for each incubation time. The general linear model was as follows.

$$Y_{ijk} = \mu + S_i + H_j + (S \times H)_{ij} + E_{ijk}$$

Where, Y_{ijk} = dependant variable, μ = general mean, S_i = plant species effect, , H_j = $_{jth}$ harvesting height effect, $(S \times H)_{ij}$ = effects due to interaction of tree species and harvesting height, and E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P \leq 0.05$. The probability of difference (pdiff) was used to compare least square means option in the lsmeans statement of SAS.

4.3.2 Correlation analysis

The Pearson's correlation coefficient was used to determine the strength of linear association between INDMD and IVND, degradation parameters and NSI according to correlation procedure of SAS (2010). Significance of correlation coefficients was declared at $P \leq 0.05$.

4.4 Results

4.4.1 Buffer solubility

Table 4.1 presents the buffer insoluble nitrogen (BIN), buffer soluble nitrogen (BSN) and buffer solubility index (NSI) of various browse leaves harvested at different heights. The results revealed that plant species × harvesting height interaction influenced ($P < 0.05$) on the buffer nitrogen solubility. From the browsable height, leaves of *E. rigida* (858.3 g/kg DM), *O. africana* (825.9 g/kg DM), *C. rudis* (807.8 g/kg DM) and *G. robusta* (799.0 g/kg DM) 9.0 g/kg DM) had the highest ($P < 0.05$) buffer insoluble nitrogen (BIN) content although *C. rudis* and *G. robusta* were similar ($P > 0.05$) to *M. capitata*. *Boscia oleoides* leaves had the lowest ($P < 0.05$) BIN content (456.1 g/kg DM) at this height.

The species and the interaction between tree species × height had a significant influence on buffer insoluble nitrogen and nitrogen solubility index, while their height did not affect these parameters. Table 4.1 shows that from the non-browsable height, *E. rigida* (860.9 g/kg N) and *O. africana* (823.3 g/kg N) leaves had the highest ($P < 0.05$) BIN content, while *B. oleoides* had the lowest ($P < 0.05$) BIN (453.6 g/kg N). *Boscia oleoides* leaves had the highest BIN at both harvesting heights. It was followed by *R. refrata* at non-browsable height with 392.88 g/kg N, which was higher ($P < 0.05$) than other browse leaves. *Olea africana* had the lowest ($P < 0.05$) BNS at both heights.

Table 4.1: Effects of browse species and harvesting height (browsable (<1.5 m) and non- browsable (>1.5 m)) on buffer insoluble nitrogen N (g/kg N), buffer soluble nitrogen N (g/kg N) and nitrogen solubility index (%) of tree leaves

	Buffer insoluble N		Buffer soluble N		N Solubility index	
Species	<1.5 m	>1.5 m	<1.5 m	>1.5 m	<1.5 m	>1.5 m
Boscia oleoides	456.1^{d}	453.6^{g}	543.9^{a}	546.4^{a}	54.4^{a}	54.6^{a}
Ziziphus mucronata	638.1^{c}	711.9^{d}	361.9^{b}	288.6^{d}	36.2^{b}	28.9^{d}
Carissa macrocarpa	643.5^{c}	659.7^{e}	356.5^{b}	340.3^{c}	35.6^{b}	34.0^{c}
Rhus refracta	644.5^{c}	607.1^{f}	355.5^{b}	392.9^{b}	35.5^{b}	39.3^{b}
Phyllanthus vessucosus	647.2^{c}	756.0^{c}	352.8^{b}	244.0^{e}	35.3^{b}	24.4^{e}
Maytenus capitata	783.1^{b}	789.2^{cb}	216.9^{c}	210.8^{ef}	21.7^{c}	21.1^{ef}
Grewia robusta	790.0^{ab}	764.2^{c}	201.0^{cd}	235.8^{e}	20.1^{cd}	23.6^{e}
Coddia rudis	807.8^{ab}	818.1^{b}	192.2^{cd}	181.9^{f}	19.2^{cd}	18.2^{f}
Oleas africana	825.9^{a}	823.3^{ab}	174.1^{de}	189.0^{f}	17.4^{ed}	18.9^{f}
Ehretia rigida	858.3^{a}	860.9^{a}	141.7^{e}	139.1^{g}	14.2^{e}	13.9^{g}
Significant level	NS		*		NS	

^{abcdefg} In a column and within harvesting height, different superscripts denote significant differences ($P < 0.05$).

NS: $P > 0.05$, * $P < 0.05$, **$P < 0.01$ and ***$P < 0.001$

SEM Standard error of the mean

Table 4.1 shows that browse species × harvesting height interaction had a significant influence on nitrogen solubility index of browse species. The NSI of leaves harvested from browsable height ranged from 14.2% (*E.rigida*) to 54.4% (*B. oleoides*). The leaves haversted at the browsable height from *Z. mucronata* (36.2%), *C. macrocarpa* (35.6%), *R. refrata* (35.5%) and *P. vessucosus* (35.3%) had a higher BSI than *M. capitata* (21.7%), *G. robusta* (20.1%), *C. rudis* (19.2) and *O. africana* (17.4%), respectively. Buffer solubility index ranged between 54.64% (*B. oleoides)* and 13.9% (*E. rigida*) for leaves harvested from the non-browsable height. *Boscia oleoides* was followed by *R. refracta* (39.28 %) leaves, which had higher (P <0.05) BSI than *Z. mucronata* leaves (28.9 %).

4.4.2 In vitro ruminal degradability

The results presented in Table 4.2 show that there was a significant (P <0.05) variation between browse species and browse species × harvesting height interaction, which had an effect on IVDMD of the leaves at 12, 24 and 36 hours of incubation. The 12h IVDMD ranged from 116.9 g/kg DM (*C. rudis* leaves harvested from non-browsable height) to 589.9 g/kg DM (*B. oleoides* leaves harvested from non-browsable height). *Coddia rudis* had the highest IVDMD (431.3 g/kg DM) amongst the leaves harvested from browsable height while *Z. mucronata* had the lowest (213.2 g/kg DM). *Boscia oleoides* (589.9 g/kg DM) leaves also had the highest IVDMD on browse leaves harvested at non-browsable height while *C. rudis* (116.9 g/kg DM) had the lowest. *Boscia oleoides* was followed by *R. refracta* (275.3 g/kg DM) then *Z. mucronata* 216.8 (g/kg DM), which was similar to C. *macrocarpa* (210.5 g/kg DM) and *G. robusta* (184.1 g/kg DM) but higher IVDMD than other leaves harvested from non-browsable height.

Table 4.2: Effects of browse species and harvesting height (<1.5 m and >1.5 m) on *in vitro* ruminal dry matter degradation (g/kg DM) of tree leaves after 12, 24, and 36 hours of incubation

	12h		24h		36h	
Browse species	<1.5 m	>1.5 m	<1.5 m	>1.5 m	<1.5 m	>1.5 m
Coddia rudis	431.3^{Aa}	116.9^{Be}	522.0^{Aa}	237.0^{Bc}	635.9^{Aa}	308.0^{Bb}
Maytenus capitata	262.4^{Ad}	138.0^{Ade}	319.4^{Acb}	193.6^{Bcd}	373.5^{Ad}	236.8^{Bd}
Olea africana	349.1^{Ab}	139.1^{Bde}	438.0^{Ab}	242.5^{Bc}	514.5^{Ab}	292.1^{Bb}
Ehreti rigida	243.6^{Ad}	156.6^{Bd}	341.2^{Ac}	179.4^{Bd}	499.7^{Ab}	199.1^{Bd}
Phyllanthus vessucosus	355.2^{Ab}	178.3^{Bd}	395.9^{Abc}	211.0^{Bcd}	462.2^{Ac}	234.1^{Bd}
Grewia robusta	253.9^{Ad}	184.1^{Bcd}	314^{Acd}	213.6^{Bc}	360.5^{Ad}	236.6^{Bd}
Carissa macrocarpa	310.1^{Ac}	210.5^{Bc}	358.9^{Ac}	216.2^{Bc}	379.5^{Ad}	250.2^{Bd}
Ziziphus mucronata	213.2^{Ad}	216.8^{Ac}	279.8^{Bd}	230.6^{Ac}	306.1^{Ae}	235.9^{Bd}
Rhus refrata	322.8^{Acb}	275.3^{Bb}	342.0^{Ac}	285.4^{Bb}	355.9^{Ad}	299.4^{Bb}
Boscia oleoides	339.5^{Bcb}	589.9^{Aa}	419.1^{Bb}	597.9^{Aa}	455.7^{Bc}	625.5^{Aa}
Significant level	***		***		***	
SEM	14.8		16.4		13.9	

^{A,B}In a row per incubation period, different uppercase superscripts denote significant differences ($P < 0.05$).

^{abcdefg}In a column and within harvesting height, different lowercase superscripts denote significant differences ($P < 0.05$).

***$P < 0.001$

SEM: Standard error of the mean

Table 4.2 shows that browse plant species, harvesting height and their interaction had a significant (P <0.05) influence on *in vitro* dry matter degradability. At the browsable height, *C. rudis* (522.0 g/kg DM) leaves had the highest (P <0.05) 24h degradability, while *Z. mucronata* had the least IVDMD (279.8 g/kg DM). *Coddia rudis* was followed by *O. africana* (438.0 g/kg DM) and *B. oleoides* (419.1 g/kg DM), *R. refracta* and *P. vessucosus*, which had similar, but higher IVDMD than other tree species. The 24h IVDMD of browse leaves harvested from non- browsable height ranged between 179.4 g/kg DM (*E. rigida*) and 597.9 g/kg DM (*B. oleoides*). The 24h digestibility value of *B. oleoides* was followed by *R. refracta* (285.4 g/kg DM), then followed by *Z. mucronata, C. macrocarpa, G. robusta P. vessucosus* and *M. capitata,* which were similar but had higher IVDMD than other browse leaves from non-browsable height. All browse leaves harvested from browsable height had higher (P <0.05) 24 h IVDMD than those sampled at non-browsable height, except for *B. oleoides. Ziziphus mucronata* leaves had the least (P < 0.05) degradability at both harvesting heights while *M. capitata* (193.6 g/kg DM) leaves were the least degradable from those harvested from the non-browsable height. After 36 hours incubation, leaves from *C. rudis* (693.9 g/kg DM) had the highest degradability, followed by *O. africana* (514.5 g/kg DM) and *E. rigida* (499.7 g/kg DM), which were similar but higher than other browse leaves harvested at browsable height while *Z. mucronata* had the lowest IVDMD. The IVDMD of leaves harvested from non-browsable height ranged between 629.5 g/kg DM (*B. oleoides*) and 199.1 g/kg DM (*E. rigida*). Degradability of leaves from *B. oleoides* was followed by *C. rudis* (308.0 g/kg DM*)* and *R. refracta* (299.4 g/kg DM), which were similar but higher (P<0.05) than other browse leaves harvested from non-browsable height.

Table 4.3 shows the dry matter degradability parameters of browse leaves harvested from different heights. It reveals variation between plant species, harvesting height and their interaction

significantly (P <0.05) influenced immediately degradable fraction (a), degradable part of the insoluble fraction of dry matter (b) and degradability rate of the dry matter (c). At non-browsable height, *B. oleoides* (291.2 g/kg DM) had highest (P < 0.05) immediate degradable dry matter fraction. It was followed by *R. refracta* which had higher '*a*' fraction compared to other browse leaves, while *M. capitata* (25.2 g/kg DM) had the lowest value. Dry matter of *R. refracta* (292.4 g/kg DM), *C. rudis* (281.2 g/kg DM) and *C. macrocarpa* (265.9 g/kg DM) leaves harvested from browsable height were rapidly degraded more than other browse leaves. *Grewia robusta* and *E. rigida* dry matter had the least amount of degradation.

Amongst the leaves harvested at non-browsable height, *E. rigida* and *B. oleoides* dry matter had the highest *b* fraction (455.8 and 382.0 g/kg DM) respectively, then *O. africana* (356.9 g/kg DM), similar (P > 0.05) to *B. oleoides* (382.0 g/kg DM) while *R. refracta* had the least 'b' fraction of 131.9 g/kg DM. The 'b' fraction of leaves harvested from browsable height ranged from 148.9 g/kg DM (*Z. mucronata*) to 728.5 g/kg DM (*E. rigida*). *E. rigida* was followed by *C. rudis* (567.9 g/kg DM), which also had higher 'b' fraction than *R. robusta* and *P. vessucosus*, which had similar but higher values than other browse leaves.

The degradation rate of the 'b' fraction of dry matter ranged between 0.131 (*Boscia oleoides*) and 0.0148 (*E. rigida*) for leaves harvested from non-browsable height. *Boscia oleoides* was followed by *C. macrocarpa* (0.069). *Boscia oleoides* leaves harvested from browsable height also had the highest (P <0.05) rate of degradation (0.037). It was followed by *Z. mucronata, C. rudis, O. africana, E. rigida, P. vessucosus* and *R. refracta*, which had similar but higher (P<0.05) 'c' value than *M capitata* and C. *macrocarpa* (0.016), which were similar and the least.

Table 4.3: Estimates of *in vitro* ruminal dry matter degradability parameters of various browse leaves harvested at two different heights.

	Degradability parameters[1]									
	a		b		c		EDMD		PDMD	
Species	>1.5 m	<1.5 m	>1.5 m	<1.5 m	>1.5 m	<1.5 m	>1.5 m	<1.5 m	>1.5 m	<1.5 m
Maytenus capitata	25.2^{Bh}	206.7^{Ad}	253.6^{Ac}	169.2^{Bf}	0.05^{Abc}	0.025^{Bb}	151.5^{Be}	246.1^{Af}	278.7^{Bd}	375.9^{Aa}
Coddia rudis	31.9^{Bh}	281.2^{Aa}	331.5^{Bbc}	567.9^{Ab}	0.038^{Ac}	0.027^{Aab}	159.2^{Bed}	479.1^{Aa}	363.4^{Bc}	849.1^{Aa}
Oleas africana	54.6^{Bg}	259.9^{Ab}	356.9^{Ab}	365.8^{Ad}	0.03^{Acd}	0.029^{Aab}	186.7^{Bdc}	390.6^{Ab}	411.5^{Bc}	625.7^{Ab}
Ehretia rigida	75.7^{Bf}	122.9^{Af}	455.8^{Ba}	729.5^{Aa}	0.0148^{Ad}	0.018^{Aab}	174.0^{Bc}	312.3^{Ae}	531.5^{Bb}	852.4^{Aa}
Grewia robusta	99.8^{Be}	128.9^{Af}	192.3^{Bcd}	461.9^{Ac}	0.056^{Ab}	0.021^{Bab}	188.8^{Bdc}	263.8^{Af}	292.1^{Bcd}	590.8^{Abb}
Phyllanthus vessucosus	111.6^{Bef}	235.9^{Ac}	314.5^{Bbc}	435.6^{Acd}	0.0169^{Adc}	0.023^{Aab}	184.2^{Bdc}	363.9^{Ac}	426.1^{Bc}	671.5^{Ab}
Carissa macrocarpa	121.7^{Bh}	265.9^{Aab}	150.0^{Bd}	322.8^{Ade}	0.069^{Ab}	0.016^{Bb}	201.8^{Bc}	337.3^{Ad}	271.6^{Bd}	588.5^{Abc}
Ziziphus mucronata	149.9^{Ac}	154.5^{Ae}	162.3^{Ad}	148.9^{Af}	0.03^{Adc}	0.035^{Aab}	212.3^{Ac}	215.2^{Ag}	312.2^{Acd}	303.5^{Ad}
Rhus refracta	219.6^{Bb}	292.4^{Aa}	131.9^{Bd}	250.3^{Ae}	0.043^{Abc}	0.021^{Bab}	272.2^{Bb}	355.0^{Adc}	351.5^{Bcd}	542.7^{Ab}
Boscia oleoides	291.2^{Aa}	254.6^{Bb}	382.0^{Aab}	269.8^{Be}	0.131^{Aa}	0.037^{Ba}	567.2^{Aa}	368.4^{Bc}	673.2^{Aa}	524.4^{Bb}
Level of significance	*		*		*		*		*	
SEM	9.27		39.04		0.01		9.96		39.95	

[A,B]In a row, for each degradability parameter, different uppercase superscripts denote significant differences ($p < 0.05$) between harvesting height

[a,b,c,d,e,f,g]In a column, different lowercase superscripts denote significant differences ($p < 0.05$) between tree species, *P <0.05

[1]Degradability parameters: a = immediately degradable fraction of dry matter; b = degradable part of the insoluble fraction of dry matter; and c = degradation rate of b fraction; EDMD = effective dry matter degradability, calculated as, $ED = a + bc/k + c$ where a, b and c are the constants from the Ørskov and McDonald (1979) equation above, and k is the outflow rate, which was assumed to be 5%/hour; PDMD = potential degradability of dry matter.

There was significant interaction between browse species × harvesting height on effective dry matter degradability. *Boscia oleoides* (567.2 g/kg DM) leaves harvested from non-browsable height had the highest EDMD. It was followed by *R. refracta* (272.2 g/kg DM), which was higher than the rest of the browse species. *Maytenus capitata* (151.5 g/kg DM) had the lowest EDMD, though it was similar to *C. rudis* (159.2 g/kg DM). At browsable height, *C. rudis* (479.1 g/kg DM) had the highest EDMD followed by *O. africana* (390.6 g/kg DM), which was higher than *B. oleoides* (368.4 g/kg DM) and *P. vessucosus* (363.9 g/kg DM), which were similar but higher than other browse species. *Ziziphus mucronata* (215.2 g/kg DM) leaves had the lowest EDMD at the browsable height. Leaves harvested from browsable height generally had higher EDMD than those harvested from non-browsable height.

Table 4.3 also shows that plant species, harvesting height and interaction between browse species × harvesting height had a significant influence on potential dry matter degradability (PDMD). The PDMD of browse leaves harvested from the browsable height was higher ($P < 0.05$) when compared to those sampled at non-browsable height for all tree species except *B. oleoides* and *Z. mucronata*. At the non-browsable height, *B. oleoides* (673.2 g/kg DM) had the highest PDMD, followed by *E. rigida* (351.5 g/kg DM). *Maytenus capitata* and *C. macrocarpa* had similar but the lowest PDMD. The PDMD of leaves harvested from browsable height ranged from 303.5 (*Z. mucronata*) to 852.4 g/kg DM (*E. rigida). Ehretia rigida* was similar to *C. rudis* (849.1 g/kg DM), which was followed by *P. vessucosus* and *O. africana*, which were similar but higher than other browse leaves.

Table 4.4 shows the effects of browse species and harvesting height on IVND of browse leaves sampled at browsable (<1.5 m) and non-browsable (>1.5 m) heights. There was browse species, harvesting height and browse species × harvesting height interaction which had a significant (P<0.05) effect on IVND of browse leaves. The leaves of *B. oleoides* harvested from both heights had the highest (P <0.05) IVND at 12, 24 and 36 hours of incubation. They were then followed by *C. macrocarpa* (302.67 g/kg N) and *R. refracta* (288 g/kg N) leaves harvested from the browsable height. Leaves of *E. rigida* (135.97 g/kg N) had the least (P <0.05) IVND. *Boscia oleoides* leaves harvested at non-browsable height had the highest IVND (608.7 g/kg N) followed by *R.refracta* (266.87 g/kg N) while *M. capitata* leaves (49 g/kg N) had the least (P<0.05) IVND. *Carissa macrocarpa*, *O. africana, M. capitata* and *C. rudis* leaves harvested from the browsable height had significantly high (P <0.05) 12h IVND compared to leaves sampled at non-browsable heigh. Only *B. oleoides* had higher 12h IVND at non-browsable height while the two harvesting heights had similar (P > 0.05) IVND for leaves from the rest of the tree species.

Table 4.4 also presents the IVND of leaves incubated for 24 hours where harvesting height had influence on IVND. *Coddia* rudis, *C. macrocarpa, E. rigida*, *M. capitata* and *P. vessucosus* leaves harvested from the browsable height had higher (P <0.05) 24 h IVND than from the non- browsable height. Results reveal that for leaves harvested at browsable height, *B. oleoides* (539.5 g/kg N) had the highest (P <0.05) 24h IVND while *Z. mucronata* had the least (P <0.05) IVND (127.6 g/kg N).

Table 4.4: *In vitro* ruminal nitrogen degradability (g/kg N) of browse plants harvested at different harvesting heights (browsable (< 1.5 m) and non-browsable (> 1.5 m)) after 12, 24, and 36h of incubation.

	12		24		36	
Species	<1.5 m	>1.5 m	<1.5 m	>1.5 m	<1.5 m	>1.5 m
Ehreti rigida	136.0^{Ae}	108.0^{Ade}	237.4^{Abc}	142.9^{Bc}	452.9^{Ab}	159.2^{Bcd}
Grewia robusta	157.1^{Aed}	131.4^{Ac}	187.7^{Ac}	156.4^{Ac}	213.5^{Ae}	186.6^{Acd}
Coddia rudis	150.6^{Aed}	70.3^{Bfe}	339.1^{Ab}	83.6^{Bd}	579.1^{Aa}	107.0^{Bd}
Maytenus capitate	144.2^{Aed}	49.0^{Bf}	202.4^{Ac}	85.1^{Bcd}	269.0^{Ade}	125.0^{Bd}
Olea Africana	161.3^{Aed}	79.9^{Be}	190.1^{Ac}	110.8^{Bdc}	354.2^{Ac}	127.4^{Bd}
Phyllanthus vessucosus	247.9^{Ac}	119.5^{Ad}	285.8^{Ab}	146.8^{Bc}	368.7^{Ac}	166.4^{Bcd}
Ziziphus mucronata	181.4^{Ad}	205.7^{Ac}	127.6^{Bd}	227.2^{Ab}	212.3^{Ae}	267.7^{Ab}
Carissa macrocarpa	302.7^{Ab}	152.9^{Bd}	316.1^{Ab}	177.2^{Bbc}	359.4^{Ac}	223.2^{Bbc}
Rhus refracta	288.0^{Ab}	266.9^{Ab}	306.3^{Ab}	262.5^{Ab}	323.4^{Acd}	269.6^{Ab}
Boscia oleodis	435.3^{Ba}	608.7^{Aaf}	539.5^{Ba}	674.6^{Aa}	605.2^{Ba}	714.6^{Aa}
Level of significance	*		*		*	
SEM	19.2		29.4		33.5	

^{A,B}In a row, for each degradability parameter, different uppercase superscripts denote significant differences ($p < 0.05$) between harvesting height

^{a,b,c,d,e,f,g}In a column, different lowercase superscripts denote significant differences ($p < 0.05$) between tree species.

*P< 0.05

SEM: Standard error of the mean

The 24h IVND of leaves harvested from the non-browsable height ranged from 83.6 g/kg DM (*C. rudis*) to 674.6 g/kg N (*B. oleoides*). *Rhus refracta*, *C. macrocarpa* and *Z. mucronata* had higher (P <0.05) IVND than other browse leaves as shown in Table 4.4 shows that the 36h IVND of leaves harvested from browsable height ranged between 212.3 g/kg N (*Z. mucronata*) and 605.2 g/kg N (*B. oleoides*). *Boscia oleoides* leaves had similar (P >0.05) 36h IVND to *C. rudis* (579.13 g/kg N) and these were followed by *E. rigida* (452.9 g/kg N). The leaves of *B. oleoides* harvested from non-browsable height, had the highest (P<0.05) 36h IVND (714.3 g/kg N) followed by *R. refracta, Z. mucronata* and *C. macrocarpa* leaves, which had higher (P <0.05) 36h IVND than the rest of the plants. The leaves of *C. rudis* had the least (P <0.05) 36h IVND (107 g/kg N). The 36h IVND of plant leaves harvested at browsable height was high (P < 0.05) when compared to leaves sampled at non-browsable height, except for *B. oleoides* leaves, which showed the opposite trend and *Z. mucronata* leaves, whose IVND was similar (P >0.05) across the two harvesting heights.

Table 4.5 presents *in vitro* ruminal nitrogen (N) degradability parameters of browse leaves harvested from two heights on the same plants. It shows that browse species, harvesting height and interaction between browse species × harvesting height had a significant influence on all degradability parameters; a, b, c, effective N degradability (END), and potential N degradability
(PND). The immediately degradable fraction of N (a) in leaves harvested from non-browsable height ranged from 27.1 g/kg DM (*M. capitata*) to 574.5 g/kg DM (*B. oleoides*). *Boscia oleoides* was followed by *R. refracta*, which was higher than *Z. mucronata* and other browse plants. *Boscia oleoides* also had highest 'a' fraction amongst leaves harvested from browsable height, it was then followed by *R. refracta*, which had a similar 'a' value to *C. macrocarpa* but higher than other browse leaves. *Grewia robusta* had the lowest immediate degradable 'a' fraction of N.

Table 4.5 shows that the 'b' fraction of leaves was influenced (P< 0.05) by browse species and harvesting height and their interaction. The results revealed that leaves harvested from browsable height ranged from 136.8 g/kg N (*R. refracta*) to 693.3 g/kg N (*G. robusta*). *Grewia robusta* had similar 'b' value with *P. vessucosus* (664.3 g/kg N) and was followed by *M. capitata* (508.9 g/kg N), which had higher (P <0.05) potentially degradable fraction than all the other browse leaves at this height. The degradable part of insoluble fraction of N for leaves harvested from non- browsable height ranged between 94.1 g/kg N (*R. refracta*) and 204 g/kg DM (*B. oleoides*). All leaves harvested from browsable height generally had a higher (P <0.05) 'b' values than those harvested at the non-browsable height.

Ehretia rigida (0.04) and *R. refracta* (0.037) had similar but high N degradability rate for leaves harvested at non-browsable height while *C. rudis, O. africana* and *P. vessucosus* had similar but least degradability rate. Amongst leaves harvested from browsable height, *C. macrocarpa* had the highest disappearance rate (0.08). It was followed by *G. robusta*, which was similar to *C. rudis* (0.04), *R. refracta* (0.04) and *O. africana* (0.03) while *P. vessucosus* N had the least rate of degradation (0.008).

Table 4.5: Estimates of in vitro nitrogen degradability parameters of various browse leaves sampled at different heights

	a		b		c		END		PND	
Species	>1.5 m	<1.5 m	>1.5 m	<1.5 m	>1.5 m	<1.5 m	>1.5 m	<1.5 m	>1.5 m	<1.5 m
Maytenus capitate	27.1^{Be}	116.7^{Ad}	164.1^{Bab}	508.9^{Ab}	0.021^{Ab}	0.02^{Ac}	75.1^{Be}	201.7^{Af}	191.3^{Bcd}	625.7^{Ab}
Coddia rudis	52.5^{Be}	107.1^{Ad}	188.9^{Ba}	438.9^{Abc}	0.016^{Bb}	0.04^{Abc}	93.5^{Be}	253.6^{Ae}	241.4^{Bcd}	546.0^{Ac}
Oleas Africana	61.9^{Be}	152.7^{Acd}	142.4^{Bab}	377.7^{Ac}	0.016^{Ab}	0.03^{Ac}	96.7^{Be}	265.2^{Ae}	204.3^{Bcd}	530.4^{Ac}
Ehretia rigida	54.8^{Be}	146.0^{Acd}	134.7^{Bab}	257.8^{Ad}	0.04^{Aa}	0.014^{Bc}	114.8^{Bde}	283.8^{Ade}	189.6^{Bd}	403.7^{Ad}
Grewia robusta	91.8^{Ade}	80.1^{Ad}	170.8^{Ba}	693.3^{Aa}	0.025^{Bab}	0.05^{Ab}	143.5^{Bd}	298.2^{Ade}	262.6^{Bc}	773.8^{Aa}
Phyllanthus vessucosus	101.1^{Bd}	167.2^{Ac}	136.5^{Bab}	664.3^{Aa}	0.017^{Ab}	0.008^{Ad}	135.0^{Bd}	317.7^{Ad}	237.7^{Bcd}	831.5^{Aa}
Carissa macrocarpa	123.0^{Bd}	249.0^{Ab}	186.8^{Aa}	167.0^{Ae}	0.027^{Bab}	0.08^{Aa}	183.3^{Bc}	320.4^{Ad}	309.8^{Abc}	416.1^{Bd}
Ziziphus mucronata	180.8^{Ac}	164.2^{Ac}	186.4^{Ba}	397.0^{Ac}	0.025^{Aab}	0.02^{Aac}	238.5^{Ab}	336.7^{Ac}	367.2^{Bb}	561.2^{Abc}
Rhus refracta	226.3^{Ab}	258.7^{Ab}	94.1^{Ab}	136.8^{Ae}	0.037^{Ab}	0.04^{Abc}	263.0^{Bb}	417.3^{Ab}	320.4^{Bb}	395.5^{Ad}
Boscia oleoides	574.5^{Aa}	394.5^{Ba}	204.0^{Ba}	393.0^{Ac}	0.033^{Aab}	0.02^{Ac}	645.4^{Aa}	517.2^{Ba}	778.5^{Aa}	787.5^{Aa}
Level of significance	*		*		*		*		*	
SEM	18.15		36.65		0.009		18.38		36.37	

^{A,B}In a row, for each degradability parameter, different uppercase superscripts denote significant differences ($p < 0.05$) between harvesting height

^{a,b,c,d,e,f,g}In a column, different lowercase superscripts denote significant differences ($p < 0.05$) between tree species.

[1]Degradability parameters: a = immediately degradable fraction of nitrogen; b = degradable part of the insoluble fraction of nitrogen; and c = degradation rate of b fraction; END = effective nitrogen degradability, calculated as, $ED = a + bc/k + c$ where a, b and c are the constants from the Ørskov and McDonald (1979) equation above, and k is the outflow rate, which was assumed to be 5%/hour; PND = potential degradability of nitrogen.

*P< 0.05; SEM: Standard error of the mean

Effective nitrogen degradability (END) of the leaves was also influenced (P <0.05) by the browse species and harvesting height and the interaction between browse species × harvesting height. Effective nitrogen degradability of the leaves harvested from non-browsable height ranged between 645.4 g/kg N (*B. oleoides*) and 75.1 g/kg N (*M. capitata*). Amongst leaves harvested from the browsable height, *B. oleoides* had the highest END, followed by *R. refracta* (417.3 g/kg N), which was also higher than all the other browse leaves at this height. *Maytenus capitata* had the least END at both heights 75.1 g/kg N (>1.5 m) and 201.7 g/kg DM (<1.5 m).

Table 4.5 also reveals significant (P <0.05) effects of browse species and harvesting height interaction between browse species × harvesting height, on potential N degradability (PND). It shows that at non-browsable height, *B. oleoides* (778.5 g/kg N) had the highest PND followed by *Z. mucronata* (367.2 g/kg N), *R. refracta* (320.4 g/kg N), and *C. macrocarpa* (309.8 g/kg N), which did not differ (P > 0.05). *Ehretia rigida* had the lowest PND (189.6 g/kg N). The PND of leaves harvested from the browsable height ranged from 395.5 g/kg N (*R. refracta*) to 813.5 g/kg N (*P. vessucosus*). *Phyllanthus vessucosus* was followed by *B. oleoides* (787.5 g/kg N), which was similar to *G. robusta* (773.8 g/kg N) but higher than all the other browse species. *Rhus refracta* (395.5 g/kg N), *E. rigida* (403.7 g/kg N) and *C. macrocarpa* (416.1 g/kg N) had the lowest level of PND.

4.4.3 Correlation analysis

The Pearson's correlation coefficients for cell wall components, IVND and NSI are presented in Table 4.6 showing that neutral detergent fibre had a significantly strong negative correlation with DMD at 36 hours of incubation while it had positive correlation for soluble phenolics. There was strong positive significant (P <0.05) correlation between nitrogen solubility index and *in vitro* ruminal N degradability at 12 (0.82), 24 (0.87) and 36 (0.91) hours of incubation. Nitrogen solubility index was positively associated with IVND, at all incubation periods.

Table 4.6: Pearson's correlation coefficient matrix for linear relationships between solubility index (SI) and *in vitro* ruminal N degradability of leaves harvested form ten browse plants at two plant heights

Chemical component	DMD12	DMD24	DMD36	IVND12	IVND24	IVND36
Neutral detergent fibre	-0.086	0.832	-0.997*	-0.377	-0.672	-0.961
Acid detergent fibre	0.021	0.886	-0.984	-0.474	-0.747	-0.926
Acid detergent lignin	0.236	0.965	-0.922	-0.653	-0.873	-0.824
Cellulose	-0.559	0.458	-0.908	0.119	-0.228	-0.974
Crude protein	0.889	0.027	0.595	-0.582	-0.269	0.746
Soluble phenolics	0.086	-0.832	0.997*	0.377	0.672	0.961
Condensed tannins	0.951	0.187	0.457	-0.706	-0.421	0.628
Solubility index				0.728*	0.597*	0.429*

DMD12, DMD24, DMD36: Dry matter degradability at 12, 24 and 36 h after incubation.

IVND12, IVND24, IVND36: Nitrogen degradability at 12, 24 and 36 h after incubation

*P <0.05

4.5 Discussion

4.5.1 Buffer solubility of browse plant leaves harvested from different heights

The buffer soluble nitrogen values obtained in this current study are in line with reports by other authors who were working with various browse plants. The NSI values observed in this study are within the range reported by Al-Masri (2013) who recorded the highest NSI of 52% for *Capparis spinose* leaves. Cudjoe & Mlambo (2014) indicated that forages with high level of solubility index are likely to be more degradable in the rumen. They planted and analysed leaves of *Gliricidia sepium*, *Leucaena leucocephala*, *Morus alba* and *Trichanthera gigantea* browse plants and reported that *L. leucocephala* had the highest NSI (20%) but they did not state harvesting period. In the current study, *B. oleoides* had the highest NSI as shown in Table 4.1, which implies that it can be the more degradable in rumen and can be used to supplement livestock fed on poor-quality diets, which are common during dry season. *Ehretia rigida* leaves had the lowest NSI at both harvesting heights indicating that it might be poorly degraded and not suitable for use as N supplement for microbes in livestock fed poor quality diet. However, the leaves may be used to increase bypass protein in high producing ruminants as the protein-tannins complex is expected to disassociate in the acidic environment of the small intestine. Apori *et al.* (1998) reported protein degradability ranging from 48.4 to 83.9% in browse leaves they analysed. The lowest NSI reported by Al-Masri (2013) was 37% for *Artemisia herba-alba* leaves, whilst Hussain *et al.* (2001) reported the lowest NSI value of 25%. In this study, *E. rigida* leaves had the lowest NSI (14.2% from browsable and 13.9% at non-browsable height), but this was higher than in *Acacia nilotica* leaves (11.5%) reported by Mnisi & Mlambo (2016) harvested at an unspecified height. At the browsable height (>1.5 m), leaves of *E. rigida, O. africana, C. rudis* and *G. robusta* had the highest insoluble nitrogen, indicating that most of protein is rumen undegradable, which could be due to their moderate to high level of tannins and phenolics, which were determined in Chapter 3, which are known to complex with the protein. This indicates that

leaves from these tree leaves might not be suitable for supplementation of poor-quality fibrous diets since they will not be able to supply sufficient quantities of rumen degradable protein to meet microbial requirements. Nitrogen Solubility index ranged between 13.9% (*E. rigida*) and 54.64% (*B. oleoides*) at non-browsable height while at browsable height it was 14.2 (*E. rigida*) to 54.4% (*B. oleoides*), much higher than the range (11.5 to 26%) reported by Mnisi & Mlambo (2016). The variation with reports by other researchers could be due to differing research location which has different biotic and abiotic factors such as genetics, environmental factors such as soil (fertility, pH, type), and season (wet and dry). Despite the fact that the sampling was done during almost same period of the year (autumn), variation on amount of rain and sunlight intensity can still lead to varying levels of the chemical composition of these browse plants. These factors tend to influence the chemical composition of the leaves, if they are at the extremes; they stress the plants, which accelerate biosysnthesis of cell wall components (cellulose and lignin) and plant secondary metabolites such as phenols and tannins (Njidda *et al.*, 2014). These metabolites can complex with N and reduce its solubility in buffer and degradation in the rumen. The major source of NSI variation among tree leaves of the current study could be genetic make up, as they grew in the same environment and were exposed to similar conditions so their response was influenced by their genetic make-up. Since plants use plant secondary metabolites such as phenols and tannins as defense mechanism, these chemicals formed complexes with protein which could not disassociate under neutral conditions. Apori *et al.* (1998) also explained that protein solubility and bio-availability is also understood to be affected by the amount of structural and non-structural protein in the feeds.

4.5.2 Simulated rumen fermentation

Njidda *et al.* (2014) emphasized the importance of investigating the digestibility of browse plants in order to estimate nutrient availability and hence supplementation requirements. The results presented in Table 4.2 show that browse species and harvesting height and their interaction influenced the IVDMD of the leaves. The degradability of the leaves from tree species varied significantly, which is in line with results by Belachew *et al.* (2013) who reported significant variation on degradability parameters of leaves from different browse species. Njidda *et al.* (2014) explained that this could be due to lignocelluloses complexes with tannins which are known to impede rumen microbial attachment and, therefore, degradation of leaves. The rate and extend to which these complexes are formed differs with the plant species (genetics) resulting in differing *in vitro* dry matter digestibility of the browse leaves. The results from the previous chapter in this thesis revealed that there were significant influences of harvesting height on condensed tannin (CT) content of all browse plant species. Formation of complexes between lignocelluloses and CTs (which varied with harvesting height), could have been the cause of harvesting height variation in IVDMD but fibre did not differ with plant height as shown in previous chapter.

After 24 hours of incubation, *C. rudis* (522.03 g/kg DM) leaves harvested at the browsable height had the highest DM degradability. According to Belachew *et al.* (2013), *in vitro* ruminal degradability of more than 60% shows that leaves are greatly degradable. This suggests that *in vitro* degradation of all browse species harvested at different heights could be low as, they had less than 60% DM degradability at 12, 24 and 36 hrs except *Coddia rudis* from non-browsable height and *B. oleoides* at browsable height. The leaves of *Z. mucronata* had higher degradability values of 279.8 g/kg DM (browsable height) and 200 g/kg DM (non-browsable height) than the 163.8 g/kg DM for same *Z. mucronata* harvested in Molelwane Farm and Masutlhe reported by Mnisi & Mlambo (2016). The differences could be due to varying location with different abiotic

conditions such as temperature, soil and rainfall and they also did not specify the height at which they sampled the browse plant leaves. However, the 24h DM degradability of browse leaves observed in this study was lower than that reported by Apori *et al.* (1998) (423 to 638 g/kg DM for, *Spondias mombin*, *Antiaris toxicaria*, *Baphia nitida* and *Ficus exasperate*) and values of *Ficus polita, Ficus thonningii, Batryospermum paradoxum, Kigalia africana, Celtis integuifolis, Khaya senegalensis, Leptadenia lancifolia* and *Ziziphus abyssinica* (630-700 g/kg DM) as reported by Njidda (2010). The differences in *in vitro* dry matter digestibility of browse leaves seen across the different studies could have been due to variation in the growth environments, which influence the chemical composition of forage, since the studies were done in different areas. Ammar *et al.* (2004) and Njidda *et al.* (2014) indicated that digestibility is mainly influenced by chemical composition of the samples, especially their cell wall content (NDF, cellulose and lignin), level of N and tannins concentration. The cell wall fractions such as ADF, ADL and cellulose may have a negative impact on digestibility of browse plants, as Ammar *et al.* (2004) reported significant negative correlation between them. Indeed, *B. oleoides, M. capitata* and *C. rudis*. *Coddia rudis* leaves had the lowest NDF as well as the highest *in vitro* ruminal degradability at all harvesting heights, which is supported by correlation analysis presented in Table 4.6 that shows that they are inversely related. This is in agreement with report of Njidda *et al.* (2014) who indicated that high digestibility is achieved mostly when NDF is less than 35%. Thus, the intake of *C. rudis* leaves can be expected to be higher than other leaves. Lignin is also one of the cell wall portions that can reduce feed degradability. Lignin is considered an indigestible portion and impedes microbial enzymes from reaching and degrading the structural polysaccharides of the cell. Forages with high levels of lignin and cellulose tend to have low digestibility as lignin acts as a physical barrier to microbial enzymes. Ammar *et al.* (2004) explained that lignocelluloses can also form complexes with tannins, which prevent microbial attachment and degradation, or by directly inhibiting celllulytic microorganisms, or both, leading to reduction of fibre degradation.

The results presented and discussed in the previous chapter showed that the browse leaves contained moderate to high CTs, ranging from 0.12 $AU_{550\ nm}$/200 mg (*B. oliedes*) to 1.04 $AU_{550\ nm}$/200 mg (*C. macrocarpa*), which could have formed complexes with lignocellulose leading to reduced IVDMD. Table 4.2 and 4.4 shows that CTs in *B. oleoides* did not negatively influence dry matter digestibility and nitrogen digestibility respectively, suggesting that the low levels of CTs have no anti-nutritional effect. The NDF and CTs in *C. rudis* had a negative association with DMD and ND. Njidda *et al.* (2014) and Aderinboye *et al.* (2016) noted that forage that is low in CP content (less than 80 g/kg DM) is poorly digestible as the protein is not adequate to meet requirements for rumen microbes. The moderate *in vitro* nitrogen digestibility observed in all the browse leaves may be due to the moderate to high fibre and tannin content of the browse plants. Acid detergent lignin in *P. vessucosus* had a negative relationship with nitrogen digestibility. Moderate nitrogen degradability may be beneficial since the browse leaves can be valuable sources of rumen-bypass protein in ruminant diets (Aganga & Mosase, 2001). Bypass protein is especially important in high-producing ruminant animals whose protein requirements tend to be much higher than what rumen microbes alone are able to supply. All browse leaves harvested from browsable height had higher 24h *in vitro* dry matter digestibility than those harvested from non-browsable height, except for *B. oleoides.* Browse plant leaves with *in vitro* degradability that is less than 60% at 24 h of incubation is considered to have low degradability. These is contrary to anticipated results, as non-browsable height is mostly new shoots that are tender, with low fibre and nutritious foliage, while browsable height would be old leaves. Alternatively it is possible that browsing encouraged or stimulated emergence of new shoots and leaves of higher quality than found at higher heights. However, the results in Chapter 3 (Table 3.1) show that harvesting height did not cause variation on some factors (cell wall components) that affect degradability (Ammar *et al.*, 2004). This is consistent with Diba & Tolera (2013) who reported that the DM degradability and its characteristics (factors a, b and c) of forage Napier grass

were not affected by cutting height. Plant growth pattern can also be taken into consideration, as some browse plants are shrubs with new shoots growing within browsable height stimulated by harvesting.

Nitrogen degradability in rumen is one of the crucial digestive processes in ruminant nutrition. Falahatizow *et al.* (2015) emphasized that estimation of ruminal protein degradability is one of the vital factors in feed evaluation systems, which is necessary to determine the nutritional requirements of ruminants (Hedqvist & Udén, 2006). It is crucial to provide ruminal degradable protein to meet the needs of rumen microbes as well as undegradable protein especially in ruminants that need high metabolisable protein (Klopfenstein *et al.*, 2001). Therefore, it is important to know the kind of protein contained in the feed offered to ruminants, hence the need for nitrogen degradability analysis of the forage. There is not much information on ND of the browse species in our current study carried out at our locations except *Z. mucronata* that has been studied by various researchers though they did not specify the sampling heights. Forage is scarce, as there are not many studies reporting on it, most of studies report only on DM digestibility. The study evaluated the nitrogen degradability (ND) of various browse species harvested at browsable and non-browsable height.

The fact that leaves of *B. oleoides* harvested from both browsable and non-browsable heights had the highest (608.73 g/kg N) nitrogen digestibility after 12 hours of incubation while *E. rigida* (135.97 g/kg N) had the lowest ND is uncommon observation. Apori *et al.* (1998) noted that differences in protein degradability are being associated with the amount of structural and non-structural protein and carbohydrate fractions, which in turn affect solubility and bio-availability. Apori *et al.* (1998) further reported a negative relationship between CP degradability with soluble phenolics. Makkar (2003) and Patra & Saxena (2009) indicated that high amounts and molecular

weight of CTs in browsable parts of plants limit the nutrient digestibility, nutrient utilization, and nitrogen (N) retention, which could be the reason of variation on ND between these browse species and harvesting heights. Most of leaves harvested at browsable height and incubated for 12 hours had higher ND than those harvested at non-browsable height, such as for *C. macrocarpa*, *O. africana, M. capitata* and *C. rudis*. This could be due to the presence of new, tender leaves as a result of continuous browsing. Only *B. oleoides* leaves had higher ND at non- browsable height while for other species had similar nitrogen digestibilityat both harvesting heights.

4.5.3 DM and N degradability parameters

The results in Table 4.3 showed that plant species, harvesting height and their interaction had a significant influence on immediate degradable fraction (a), degradable part of the insoluble fraction of dry matter (b) and degradability rate of the dry matter (c). The immediately dry matter degradable fraction 'a' was generally low across the leaves of different browse species and harvesting heights studied, though lower than for other browse leaves reported by other authors such as Njidda & Olatunji (2012) who reported 75% for *Ziziphus abyssinica* and *Ziziphus mauritiana*. Low results could have been due to the presence of secondary plant metabolites which are known to form complexes with other nutrients including protein, leading to reduced degradability of such a nutrient. Njidda & Olatunji (2012) reported range of 8.01% (*Ziziphus abyssinica*) to 17.11% (*Ziziphus mauritiana*) for soluble dry matter fraction 'a' while Apori *et al.* (1998) reported 14.3% (*Antiaris toxicaria*) to 34% (*Gliricidia sepium*) of leaf samples from some Ghanaian browse plants. The soluble dry matter fraction 'a' of current study ranged between 25.2 g/kg DM (*M. capitata*) harvested from non-browsable height and 292.4 g/kg DM (*R. refracta*) from browsable height. All browse plants were harvested from browsable height except *Z. mucronata* (154.5 g/kg DM) in browse leaves reported by Njidda & Olatunji (2012) but within the range reported by Apori *et al.* (1998). This could be related to the loss of finer particles from

the bags in this treatment and mechanical action rather than a greater solubility (Belachew *et al.*, 2013). The differences on 'a' fraction between browse species harvested at the same heights could be due to genetic variability of the tree species since they were exposed to similar environmental conditions, so their response will be influenced by their genetic make-up. Mupangwa *et al.* (2003) explained that differences in effective degradability of dry matter in forages closely resemble the proportion of potentially degradable dry matter and level of NDF, which differed between browse plants but was not influenced by harvesting height. Singh & Makkar (1992) indicated that differences may be due to relations with fibrous components such as the cellulose and hemicellulose. Llamas-Lamas & Combs (1990) also noticed an inverse relationship between fibre and effective DM degradability in browse leaves. They observed that forages with low fibre had high effective DM degradability while those with high fibre content had low effective DM degradability. Acid detergent fibre and lignin of the browse leaves of the current study were high, which could be the reason of low 'a' fraction in some plants. Njidda & Olatunji (2012) observed that the rapidly degradable fraction 'a' was generally low across the leaves studied and suggested that it could be an indication of high levels of lignifications in most of the leaves. Other factors that could have led to variation in the 'a' fraction between the present study and others could be due to genetic variation and location (Belachew *et al.,* 2013). This tends to affect the chemical composition such as fibre and N accumulation, which in turn affect dry matter degradability.

Leaves harvested from non-browsable height had higher 'a' fraction than those from browsable height despite that in Chapter 3, Table 3.1 shows that factors affect 'a' fraction (NDF, ADF, ADL and cellulose) were not affected by harvesting height. Despite the high 'b' fraction value obtained for *E. rigida* (728.5 g/kg DM), leaves from these plants generally had low 'b' fraction. Only *E. rigida* was within the range reported by Njidda & Olatunji (2012) who observed a range of 651.9 g/kg DM (*Z. abyssinica)* to 758.1 g/kg DM (*Z. mucronata*). Amongst the leaves harvested at

non-browsable height, *E. rigida* (455.8 g/kg DM) had the highest 'b' fraction of dry matter, while *R. refracta* (131.9 g/kg DM) had the lowest 'b' fraction and was lower than the lowest (651.9 g/kg DM) reported by Njidda & Olatunji (2012). Belachew *et al.* (2013) reported '*b*' fraction values of leaves, which ranged between 145.5 g/kg DM (*Maesa lanceolata*) and 162.0 g/kg DM (*Ekebergia capensis*), while Apori *et al.* (1998) reported a range from 68.0 g/kg DM (*Antiaris toxicaria*) and 253.0 g/kg DM (*Baphia nitida*). Belachew *et al.* (2013) suggested that these differences could have been caused by differences in plant species, location and concentrations of nitrogen and secondary plant components, especially condensed tannin.

The degradability rate of 'b' fraction of dry matter (c) ranged between 0.0148 (*E. rigida*) and 0.131 (*Boscia oleoides*) for leaves harvested from non-browsable height. Tolera *et al.* (1997) reported a range of 0.06-0.089 for browse species at various stages of growth. On the other hand, Apori *et al.* (1998) recorded disappearance rates of the 'b' fraction between 0.0433 (*S. mombin*) and 0.125 (*Griffornia simplicifolea*). This indicates that the disappearance rate of slowly degradable fraction for the current study was comparable to that of other reported studies. *Boscia oleoides* (567.2 g/kg DM) leaves harvested from non-browsable height had the highest effective dry matter degradability, while *Maytenus capitata* (151.5 g/kg DM) had the lowest EDMD. At browsable height, *C. rudis* (479.1 g/kg DM) had the highest EDMD, while *Z. mucronata* (215.2 g/kg DM) had the lowest ED. Effective dry matter degradability of the current study was within the range reported by Njidda & Olatunji (2012) and Ozkan & Sahin (2006). Ozkan & Sahin (2006) reported EDMD for various oak tree species, which ranged from 49.8% (*Q. coccifera*) to 55.3% (*Q. branti*) while Njidda & Olatunji (2012) reported lower levels of 29.50 (*Ziziphus abyssinica)* to 22.60 (*Ziziphus spinachristi)* at 0.12 outflow rate. Leaves harvested from browsable height generally had higher EDMD than those harvested from non-browsable height.

Boscia oleoides and *E. rigida* were the only browse species with leaves which had PDMD above 50% at non-browsable height as shown in Table 4.3. Low levels of PDMD could be due to moderate to high concentrate of fibre, soluble phenolic and condensed tannins in the browse leaves at both heights as shown in Chapter 3. The potentially degradable DM value of browse leaves harvested from browsable height was more than the 73.20% reported by Njidda & Olatunji (2012) but within 50.6% (*Baphia nitida*) and 88.4% for *T. populnea* reported by Apori *et al.* (1998). Tolera *et al.* (1997) on the other hand reported PDMD ranging between 75.9-80.7% of browse species they analyzed at various stages of growth. The variation could be due to varying genetic components of the different species, different growth environment, stage of growth although most of researches did not state the height they sampled at, and herbivory, but they did not affect the chemical composition and utilization of browse leaves (Al shafei & Nour, 2016).

Harvesting leaves at browsable height resulted in higher PDMD than leaves obtained from non-browsable height for all tree species except *B. oleoides* and *Z. mucronata,* despite non significant variation in cell wall componets, phenolics between these heights, which is one of the the main factor expected to affect degradability. Ozkan & Sahin (2006) indicated that browse leaves contain various levels of tannins which complexes with cell wall components and cell proteins and therefore affect degradability depending on tannin properties and the strength of bonds. The results of chapter 3, show that on average, tannin content of leaves at non-browsable height (0.61 $AU_{550\ nm}$/200 mg) was higher than at browsable height (0.55 $AU_{550\ nm}$/200 mg). This could relate to PDMD, browsable height had higher PDMD as it has lower CTs than those from non- browsable height for all tree species except *B. oleoides* and *Z. mucronata*. The harvesting height had some significant influence on CTs content in the browse leaves, as leaves of *C. rudis, G. robusta* and *Z. mucronata* harvested at non-browsable height had higher condensed tannin content than those

harvested at browsable height. Ogunbosoye & Babayemi (2010) also noted that CTs can lower dry matter digestibility.

4.5.4 Nitrogen degradability parameters

Different browse plant leaves had different immediate degradable N fraction (a), but leaves harvested from non-browsable height were generally higher than leaves harvested from browsable height. This is so despite the fact that condensed tannins content in the browse leaves, at non-browsable were higher than at browsable height and tannins are known to form complexes with proteins. Suggestion could be that these tannins were free tannins which were also soluble. Various researchers such as Apori *et al.* (1998) and Mlambo & Cudjoe (2014) investigated the 'a' fraction of different browse species and reported varying observations, though they did not indicate height at which the leaves were harvested. Apori *et al.* (1998) investigated *in sacco* degradability of leaf samples from some Ghanaian browse plants and reported *Antiaris toxicaria* as having the lowest 'a' value of 95 g/kg N while *Baphia nitida* had the highest value of 332 g/kg N. On the other hand, Cudjoe & Mlambo (2014) reported that the rapidly fermentable N fraction 'a' was highest in *M. alba* leaves (734.9 g/kg N) and least in *T. gigantea* leaves (139.5 g/kg N). The difference between leaves of the current study would be due to genetics, while variation with other researcher's results could be due to both genetics and location as environmental factors differed, resulting on varying chemical composition which has influence on degradability of plant leaves.

Ehretia rigida (0.04) had the highest disappearance rate of the 'a' fraction for leaves harvested at non-browsable height while *P. vessucosus* had the least disappearance rate of the 'a' fraction. Amongst leaves harvested from browsable height, *C. macrocarpa* (8%/hour) was within the range reported by Apori *et al.* (1998) on 'b' fraction of *Griffornia simplicifolea*, whose rate of

degradation was 10.23%/hour. The rate of degradation of *P. vessucosus* leaves (0.008) was the lowest. Promkot *et al.* (2007) emphasized that the rate and extent of protein degradation in the rumen is very crucial, as it determines the amount of nitrogen available to microorganisms and amino acids at the small intestine. Promkot *et al.* (2007) suggested that high levels and rates of degradability of CP could be due to the structure and solubility characteristics of protein in feed which could be easily attacked by microorganisms in the rumen. They suggested that high amount of undegradable protein can lead to reduced effective DM degradability in cassava hay (47.5%) which contained high undegradable CP.

One of the vital aspects of feed evaluation systems is estimation of ruminal protein degradability, as it reveals the amount of nitrogen available to ruminal microbes. Van Amburgh *et al.* (2012) explained that consumed protein has at least three fates in the rumen: it is degraded by rumen microbes to ammonia and utilized for bacterial protein synthesis, absorbed through rumen walls as ammonia and converted to urea in the liver, or escapes microbial action and becomes metabolizable protein directly in small intestine. Oh *et al.* (2008) explained that ingested protein is subjected to microbial attack in the rumen and extensively degraded into peptides and, subsequently, amino acids and ammonia that are mainly used for microbial protein synthesis. The rate and extent of protein degradation depends on the proteolytic activity of the ruminal microflora and the type of protein (susceptibility and accessibility of peptide bonds). If protein degradation is rapid, more feed nitrogen (N) is degraded to ammonia than the quantity required for optimal microbial protein synthesis which can be wasted as some will be excreted in urine as urea (Broderick & Albrecht, 1997). This shows the need to know the amount of rumen degradable and undegradable protein in the browse plant leaves for efficient utilization of the available feed.

Jančík *et al.* (2010) noted that the rate and extent of DM fermentation in the rumen are crucial determinants of the nutrients utilized by ruminants. Falahatizow *et al.* (2015) reported that the amount of dry matter disappearing at the end of the incubation in their study was significantly influenced by the chemical composition of the feed. The main factor influencing the rate of fermentation of feeds is the structure of the carbohydrate fraction, especially the extent of lignification of the cell wall (Nagadi *et al.*, 2000). High amounts of structural cell wall fractions in feed will result in extended period in the rumen, while non-structural fibre will take a shorter degradation. Structural carbohydrate that has bonded with tannins and protein may lead to reduced supply of N to rumen microbes, hence the need to supplement such diet with rumen degradable protein.

All browse leaves except *B. oleoides*, harvested from non-browsable height had lower END than the low value of 344.3 g/kg DM obtained for *Trichanthera gigantean* (Cudjoe & Mlambo, 2014). Amongst leaves harvested from browsable height, *B. oleoides* leaves (517.2 g/kg DM) had the highest END, while *M. capitata* leaves (201.7 g/kg DM) had the least END. Cudjoe & Mlambo (2014) reported that END ranged between 344.3 g/kg DM (*T. gigantean)* and 809.0 g/kg DM (*Gliricidia sepium*). The variation between the browse leaves of the current study and other studies could probably be due to the varying chemical composition, which is mainly influenced by biotic (genetic, harvesting height and herbivory) and abiotic factors (location, climatic condition (temperature, rainfall intensity, distribution, soil: type, fertility and pH) (Al shafei & Nour 2016). Promkot *et al.* (2007) reported low effective CP degradability of cassava hay (47.5%) and implicated high rumen bypass protein, suggesting that the END observed in the current study was low and would be source of undegradable protein.

Leaves of all browse species harvested from browsable height had higher PND than those harvested from non-browsable height. Some of browse leaves (*G. robusta* with 693.3 g/kg N) harvested from browsable height had PND falling within the range of 403.0 g/kg N and 832.0 g/kg N reported by Apori *et al.* (1998). Table 4.5 shows that the PND of most browse leaves harvested from browsable height was less than the range reported by Apori *et al.* (1998), who obtained the lowest value of 403.0 g/kg N for *B. nitida*, suggesting that most of the browse plant leaves can supply rumen microbes with over half of nitrogen content All leaves harvested from browsable height generally had higher potentially degradable fraction of N than non-browsable height, which may be due to protein-tannin complexes which would slow degradation of N in rumen thus supplying the rumen bypass protein (Promkot *et al.*, 2007). Results in Chapter 3 showed that at non-browsable tannin levels (0.61 $AU_{550\ nm}$/200 mg) were higher than browsable height (0.55 $AU_{550\ nm}$/200 mg), which could mean that more N was interwoven with CTs leading to increased PND.

4.5.5 Association between solubility index (NSI) and in vitro ruminal N degradability of leaves

The strong positive correlation between solubility index and *in vitro* ruminal N degradability at 12 (0.82), 24 (0.87) and 36 (0.91) hours of incubation (Table 4.6) is contrary to results reported by Cudjoe & Mlambo (2014). These researchers reported no association between SI and IVND at 12, 24 and 48 post-incubation for all the browse plant leaves they analysed. The current study, in Table 4.6, shows that cell wall components (NDF, ADF, ADL and cellulose) had no association with DMD at 36 hrs and ND at 12, 24 and 36 hrs of incubation. Costa *et al.* (2016) indicated that tannin can reduce ruminal degradability of the dry matter and fibrous fractions by forming complexes with protein and fibre, which reduces rumen microbial attachment and degradation.

4.6 Conclusions

The IVDMD of leaves harvested from both browsable and non-browsable heights was generally low. *Boscia oleoides* had the highest IVND and NSI followed by *Z. mucronata*, *C. macrocarpa, R. refracta* and *P. vessucosus* indicating that they can be used as supplements to meet microbial N requirement during dry season, when the quality of pasture is poor and fibrous. *Boscia oleoides* also had the highest immediate degradable 'a' fraction of N of leaves harvested from both heights. Leaves of *P. vessucosus, M. capitata*, *G. robusta* and *C. rudis* harvested from browsable height had higher 'a' fraction than from non-browsable, indicating that leaves frombrowsable height emphasizing their potential, use as crude protein supplement for livestock fed poor fibrous diets. Harvesting height influenced IVDMD and IVND, but did not affect NSI. The leaves harvested from browsable height had higher IVDMD and IVND than those harvested at non-browsable height, suggesting that they can provide N to support rumen microbiota than those from non-browsable height. Other browse plants such as *E. rigida* can be used to supplement microbial protein in high producing livestock, as their IVND and NSI were low, indicating that their protein is rumen bypass hydrolysable in small intestine. It can also be concluded that NSI is a poor predictor of *in vitro* ruminal N degradability for all browse leaves except for *P. vessucosus.*

4.7 References

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5 CHAPTER FIVE: PREDICTION OF THE NUTRITIVE VALUE OF TREE LEAVES USING NEAR INFRARED REFLECTANCE SPECTROSCOPY

Abstract

The purpose of this research was to evaluate near infrared reflectance spectroscopy (NIRS) technology as a possible procedure for prediction of nutritive value of browse tree leaves sampled in Eastern Cape Province of South Africa. Leaves from ten browse trees (*Maytenus capitata, Olea africana, Coddia rudis, Carissa mucrocarpa, Rhus refracta, Ziziphus mucronata, Boscia oliedes, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida*) were harvested from two browsing heights (< 1 m and > 1.5 m), dried and ground (1 mm sieve). SpectraStar XL was used to scan (32 per sprectra at 1100 to 2500 nm) leaf samples at 2 nm intervals. The generated spectra were recorded in diffused reflectance as log (1/R). The samples were then analysed for chemical components, buffer-soluble nitrogen and *in vitro* ruminal dry matter and N degradation to generate reference values. Reference values were transferred into NIRS spectral data file where UCal software (Unity Scientific, Australia) was used to generate calibration equations. Generated calibration equations were externally validated with the aid of reference values and respective spectral data from an independent set of browse tree leaves from *Z. mucronata, Acacia erioloba* and *Acacia nilotica* trees harvested from Masuthle and Molelwane communal areas in the North-West province of South Afraica. Results showed that all chemical parameters had good calibration statistics with high (> 0.8) R^2 values. However, calibration models for condensed tannins and soluble phenolics (SPh) had R^2 values of 0.92 and 0.40, respectively. Calibration models had a high prediction ability (0.89) and lowest standard error of correlation (41.58) for BINSN. For DMD12 h R^2 value for the calibration model was 0.46, while its SEC was 53.16 after 12 h of incubation, R^2 = 0.37; SEC = 65.01 after 24 h of incubation, and R^2 = 0.27; SEC = 106.59 after 36 h of incubation. Spectral data predicted high (81%) variation in ruminal N

degradability at 12 h with the lowest SEC (58.26). External validation revealed that the prediction accuracy of calibration model for total N level was high since it was able to explain 88% of the variation in this parameter in an independent samples and had a small standard error of prediction (SEP) of 16.34. The calibration model for organic matter, similarly, had a high R^2 value (0.65) when externally validated. However, validation statistics were poor for NDF (R^2 = 0.37, SEC = 355.01) and ADF (R^2 = 0.11, SEC = 154.94). The results show that only the OM and N calibration models can precisely be used to predict these components in browse plants leaves in general. This suggests that the NIRS technique can be used to rapidly predict total N and OM content of browse leaves that are frequently used as protein supplements.

Key words; Near infrared spectroscopy, browse plant leaves, prediction, calibration, validation

5.1 Introduction

Browse plants play a crucial role in livestock feeding, especially during dry seasons when the quantity and quality of forage has declined but they have received little attention. Their leaves are able to retain their nutritional value throughout the dry season when grasses dry up and deteriorate both in quality and quantity. Knowledge and understanding of the nutritional value of these browse plant leaves can help farmers especially when supplementing their livestock. Indeed, Goedhart (1990) emphasized that for adequate feeding of livestock, farmers need information on the nutritive value of the available feedstuffs. The NIRS technique can be used for the quick analysis of the browse plants but it needs to be calibrated and validated first since a large number of non-conventional feedstuffs have not yet been characterized using NIRS (Windham *et al.*, 1989). Givens *et al.* (1997) indicated that this technique can make several analyses concurrently and it is simple to use and operate. Landau *et al.* (2006) indicated that for models developed using NIRS technique to be precise, samples whose results will be used as reference values for calibrations of NIRS should be analysed with comprehensive analytical procedures. Therefore, it is vital to create reliable and precise data sets to serve as reference value for the calibration of NIRS.

The near infrared region (1200 to 2500 nm) contains information concerning relative proportions of C–H, N–H, and O–H bonds, the primary constituents of the organic molecules such as protein, cellulose and lignin in forages (Morris, 1989; Ramirez *et al.*, 2015). Coblentz (1905) indicated that various chemical bonds differ in strength and hence the amount of energy required for the bond vibrations to move from one level to the next in different forage samples. The variation in energy is reflected by differing spectra as a series of absorptions at different wavelengths (Cozzolino & Moron, 2004). These absorptions are due to interactions with the fundamental vibrations of the chemical bonds associated with the atoms of the groups.

Dryden (2003) and Ramirez *et al.* (2015) explained that diffused reflection carries an information that identifies chemical bonds within the sample, such includes –CH, -OH, -NH and –SH bonds enabling the identification of sugars, structural carbohydrates, proteins, 121 lipids and their concentration.

Near infrared reflectance spectroscopy technique can be used as an alternative to traditional and conventional analyses, which are used for nutritional evaluation of feeds and very few studies have been carried out with non-conventional feedstuff such as phenolic-rich browse tree leaves (Foley *et al.,* 1998). It is one of the latest technologies that is efficient (cost-effective and fast), accurate, non-destructive and environmentally friendly feed analysis procedure (De Boever *et al.*, 1995; Swart *et al.,* 2012; Ramirez *et al.,* 2015). Huang *et al.* (2008) indicated that it is the most efficient and advanced tool that has been used for feed analysis. This technique offers an alternative, applicable procedure for evaluation of chemical composition including secondary plant metabolites such as phenolics in feed material (Windham *et.al.,* 1989). The technique can also be used for predicting *in vitro* ruminal degradability of feeds (Landau *et al.*, 2006), which will eliminate the laborious procedure of cannulating animals and *in sacco* degradability. The efficacy, precision and rapidity of this method enables fast analysis of feedstuff and helps to close nutritional knowledge gap of browse plants especially in the Eastern Cape province.

Hence, the purpose of this research was to calibrate and validate NIRS for estimation of chemical composition and *in vitro* ruminal degradability of *Maytenus capitata, Olea africana, Coddia rudis, Carissa macrocarpa, Rhus refracta, Ziziphus mucronata, Boscia oleoides, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida* leaves harvested from Eastern Cape Province of South Africa.

5.2 Materials and methods

5.2.1 *Near infrared reflectance spectroscopy*

Leaf samples were harvested from *M. capitata, O. africana, C. rudis, C. mucrocarpa, R. refracta, Z. mucronata, B. oleoides, G. robusta, P. vessucosus* and *E. rigida* trees and processed as described in Chapters 3 and 4. They were dried at 60 °C and milled (Polymix PX-MFC 90 D) through a one mm sieve. Spectral data were generated by scanning the powdered leaf samples using a Spectrastar XL (Unity Scientific, Australia) within a wavelength range of 1100 to 2500 nm (near infrared) at 2 nm intervals (Corson *et al.*, 1999). Energy in this spectral range was directed onto the leaf sample and the reflected energy (R) was estimated by the instrument.

5.2.2 *Calibration and validation*

The calibration of NIR Spectroscopy was done by associating the generated spectra with chemical composition as well as the *in vitro* ruminal fermentation parameters of the of the plant leaves. The chemical composition and *in vitro* ruminal reference data on plant leaves were generated in the laboratory as described in Chapters 3 and 4. Reference values were transfered into NIRS spectral data file where UCal software (Unity Scientific, Australia) was used to analyse and generate calibration equations. The NIRS calibration equations were determined for organic matter, neutral and acid detergent fibre (NDF and ADF); acid detergent lignin (ADL); buffer soluble nitrogen; dry matter digestibility (DMD) at 12, 24 and 36 hours; nitrogen degradability (ND) at 12, 24 and 36 hours; immediate degradable 'a' fraction of DMD and ND; degradable part of the insoluble 'b' fraction of DMD and N, degradation rate of 'b' fraction, Effective DM (EDMD) and N degradability (END); and potential dry matter degradability (PDMD) and nitrogen degradability NDa, NDb, NDc, END and PND for leaves from *Maytenus capitata, Olea africana, Coddia rudis, Carissa mucrocarpa, Rhus refracta, Ziziphus mucronata, Boscia oleoides, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida* trees.

Generated spectral data were changed into an Indico pro-format and exported to UCal software for multivariate data analysis. The first analysis in UCal software was principal component analysis (PCA) then generation of the partial least square (PLS) regression models. The first principal components from the spectral data was derived with PCA, which was testing possibility of grouping of samples and determine the possibility of spectral outliners prior generation of PLS regression equations using the data set. The optimum number of the PLS calibration equations was determinated according to Martens & Naes (1989), whereby cross-validation is explained by the prediction residual error sum of squares function to avoid over-fitting of the models. Gap-segmant 122 transformation and smoothing (5 Gap size and 7 Segment size points) were used to establish second derivative. The purpose of second derivative was to serve as a mathematical treatment, which aided in correcting the baseline effects and separation of overlapping peaks during development of calibration (Naes *et al.*, 2002). The removal of scatter effects from spectral data was done by application of standard normal variation (SNV) transformation after second derivative. Coefficient of determination in calibration (R^2) and standard error of calibration (SEC) were also calculated during statistical calculations of the calibration models. The accuracy of calibration was determined according to the coefficient of determination (R^2), which signifies the amount of variation in the reference data accounted for by the NIRS regression equation. The variation between the estimated and reference value is refered to as SEC. The preciseness of calibration model was determined through external validation whereby calibration equations were validation with independent data set of *Acacia nilotica, Ziziphus mucronata* and *Acacia erioloba* leaves sampled from trees growing in two distinct environments (Masuthle and Molelwane) in the Bokone Bophirima province of South Africa. The external validation created extra NIRS performance values for each of nutritive parameter, which included R^2 as well as standard error of prediction (SEP). The average of root mean square root mean square of the variation in terms of reference as well as predicted values when the NIRS equation is used on an independent data set

is the standard error of prediction.

5.3 Statistical analysis

The linear relationship between reference and predicted values of various nutritional components was determined via linear regression analysis using Microsoft Excel. The UCal NIRS Calibration software (Unity Scientific, Australia) was used to generate the data and spectra utilized for calibration and prediction.

5.4 Results

Table 5.1 presents calibration statistics of OM, Ash, NDF, ADF, ADL, Cellulose, N, SPh, CTs content of browse plant leaves. The results show excellent predictive ability of NIRS with very high R^2 for OM (0.97), N (0.98), NDF (0.97), ADF (0.94), CTs (0.92) and cellulose (0.8) in leaf samples.

The calibration model was able to explain 92% of the variation in condensed tannins content of tree leaves compared to the 40% observed for SPh, which indicate poor predictive ability of spectral data on soluble phenolics. The acid detergent lignin calibration model had a moderate R^2 value of 59%.

Table 5.2 shows the calibration statistics buffer soluble nitrogen and *in vitro* ruminal DM and N degradability, expressed on a DM basis. Calibration model for BINSN had a high R^2 value (0.89) and low standard error of calibration (41.58), while R^2 value for BSN was also high (0.88) with an SEC of 42.44. For DMD12 h R^2 value for the calibration model was 0.46, while its SEC was 53.16 after 12 h of incubation, $R^2 = 0.37$; SEC = 65.01 after 24 h of incubation, and $R^2 = 0.27$; SEC = 106.59 after 36 h of incubation. Spectral data predicted high (81%) variation in ruminal N degradability at 12 h with the lowest SEC (58.26). After 24 h of incubation the R^2 was 0.82 and SEC was 62.29, however, the prediction accuracy for ND36 was low ($R^2 = 0.34$, SEC=141.46).

Table 5.1: The NIRS calibration statistical data for chemical composition (g/kg DM) of browse leaves

	[1]Chemical components								
	OM	Ash	NDF	ADF	ADL	Cellulose	N	SPh	CTs
N	96	94	95	94	95	96	97	93	99
Min	788.3	47.4	262.1	162.2	90.0	10.5	79.6	455.7	0.02
Mean	856.9	79.9	464.3	274.9	153.6	121.1	114.6	694.2	0.58
Max	902.7	122.2	736.7	373.2	257.5	175.1	172.1	823.4	1.1
SD	26.53	19.4	130.7	59.6	32.88	36.57	23.85	66.16	0.36
SEC	4.48	1.812	24.18	13.52	20.95	16.55	3.44	51.37	0.11
R^2	0.97	0.99	0.97	0.94	0.59	0.8	0.98	0.4	0.92
SECV	5.05	2.44	32.29	17.18	23.37	19.5	3.92	58.1	0.12
R^2V	0.96	0.98	0.94	0.9	0.4	0.63	0.94	0.25	0.87

[1]Chemical components: OM = Organic matter; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; N = nitrogen; SPh = Soluble Phenolic; CTs = Condensed tannins.

SD; Standard deviation, SEC; standard error of calibration (SEC), R^2= Coefficient of determination in calibration SECV= Standard error of calibration validation, R^2V = Coefficient of determination in calibration

Table 5.2: NIRS calibration statistical for buffer nitrogen solubility (BINSN and BSN) and *in vitro* ruminal dry matter and nitrogen degradability of browse trees leaves

	[1]Chemical components							
	BINSN	BSN	DMD12	DMD24	DMD36	ND12	ND24	ND36
n	99	99	59	60	60	60	59	60
Min	429.5	89.5	118.5	159.0	133.1	32.0	74.9	87.5
Mean	714.83	285.8	275.42	333.34	382.02	199.83	235.04	304.22
Max	910.5	570.5	435.7	544.7	730.00	642.5	697.2	734.8
SD	123.07	122.79	71.36	81.91	124.85	133.7	174.55	173.91
SEC	41.58	42.44	53.16	65.01	106.59	58.26	62.29	141.46
R^2	0.89	0.88	0.46	0.37	0.27	0.81	0.82	0.34
SECV	50.22	51.53	62.9	72.59	120.79	71.63	81.69	154.16
R^2V	0.82	0.82	0.11	0.17	0.1	0.67	0.68	0.17

[1]Chemical components: BINSN = Buffer insoluble nitrogen, BSN = Buffer soluble nitrogen, DMD 12 = Dry matter digestibility at 12 h of incubation of incubation; DMD12 = Dry matter degradability at 12 h of incubation; DMD24 = Dry matter digestibility at 24 h of incubation; DMD36 = Dry matter degradability at 36 h of incubation; ND12; nitrogen degradability 12 h of incubation; ND24; nitrogen degradability 24 h of incubation; ND36; nitrogen degradability 36 h of incubation.

Table 5.3 presents the NIRS calibration for DMD and N degradability parameters; a, b and c, as well as their effective and potential degradability, expressed on a DM basis. Calibration models for immediately degradable fraction (a) and degradability rate of the dry matter (c) had R^2 values below 0.5 while for degradable part of the insoluble fraction of dry matter (b) of the leaves the R^2 value was 0.69. Spectral data explained 45% of the variation in effective degradability whereas potential DMD prediction was poor (0.21). The NIRS calibration statistics for N degradability are also presented in Table 5.3. It shows that prediction of immediately degradable N fraction (a) was high (0.82) with its SEC of 55.86 while R^2 for degradable part of insoluble N was poor (0.17) with a large SEC of 157.2.

Table 5.3: NIRS calibration statistical for *in vitro* ruminal dry matter degradability parameters (a, b, c, ED and PD) and in vitro ruminal nitrogen degradability (ND2a, NDb, NDc, END and PND) of leaves from browse trees

	Degradability parameters[1]									
Statistical data	a	b	c	ED	PD	NDa	NDb	NDc	END	PND
Samples	60	59	54	60	60	60	59	58	60	58
Min	16.65	91.32	0.01	133.67	246.9	13.73	71.07	0.01	66.86	173.2
Mean	169.2	323.4	0.03	281.48	491.8	166.5	273.0	0.03	260.0	437.8
Max	302.3	810.9	0.06	573.44	922.8	607.3	747.0	0.08	659.0	878.0
SD	88.92	163.68	0.01	112.31	187.4	130.0	172.1	0.01	145.5	211.5
SEC	68.65	90.85	0.01	83.56	166.9	55.86	157.2	0.01	92.02	177.1
R^2	0.4	0.69	0.17	0.45	0.21	0.82	0.17	0.14	0.6	0.3
SECV	77.93	125.91	0.01	92.48	190.6	63.64	176.1	0.01	107.4	184.9
R^2V	0.17	0.4	0.01	0.25	0.036	0.72	0.01	0.01	0.42	0.11

[1]Degradability parameters: a = immediately degradable fraction of dry matter; b = degradable part of the insoluble fraction of dry matter; and c = degradation rate of b fraction; EDMD = effective dry matter degradability, calculated as, $ED = a + bc/k + c$ where a, b and c are the constants from the Ørskov and McDonald (1979) equation above, and k is the outflow rate, which was assumed to be 5%/hour; PDMD = potential degradability of dry matter.

NDa = immediately degradable fraction of nitrogen; NDb = degradable part of the insoluble fraction of nitrogen; and NDc = degradation rate of b fraction; END = effective nitrogen degradability, calculated as, $END = a + bc/k + c$ where a, b and c are the constants from the Ørskov and McDonald (1979) equation above, and k is the outflow rate, which was assumed to be 5%/hour; PND = potential degradability of nitrogen.

NIRS statistics for degradability rate of the 'b' fraction (R^2 = 0.14 and SEC = 0.011) and potential degradability of N (R2 = 0.3 and SEC = 92.01) were less than 50% while the R^2 of effective N degradability (0.6) was moderate with SEC being 177.06.

Table 5.4: Statistics of validation for NIRS calibrated data set using external independent spectral data

	[1]Validated Parameters			
	OM	NDF	ADF	N
n	49	50	47	50
Ave Ref	824.62	509.81	421.47	2.35
Ave. NIRS	862.68	501.0	302.86	18.28
Difference	88.07	8.81	118.61	-15.92
Slope	1.48	-0.15	0.24	0.16
R^2	0.65	0.37	0.10	0.88
SEP	52.53	355.01	154.94	16.34
SEP (C)	36.2	354.9	99.69	3.66
AVE. GD	1.51	3.3	1.88	1.82
AVE. ND	0.78	2.03	1.00	1.07

[1]Validated Parameters: OM = Organic matter; NDF = neutral detergent fibre; ADF = Acid detergent fibre; SEP= Standard error of prediction N = Nitrogen, n = Sample size

Figure 5.1 compares modelled values with reference values for validated independent samples of chemical components for browse plant leaves. The regression plots reveal that total N had the relatively high R^2 value (0.88) and the low SEP (16.34) revealing excellent capability of NIRS on N prediction in browse plant leaves. The model for organic matter prediction had a good R^2 value (0.65) and moderate SEC (52.53). However, Figure 5.1 also shows that low R^2 values were obtained for validated data of NDF (0.37) and ADF (0.11) while their SEC was high (355.01 and 154.94, respectively) in the independent sample of browse leaves.

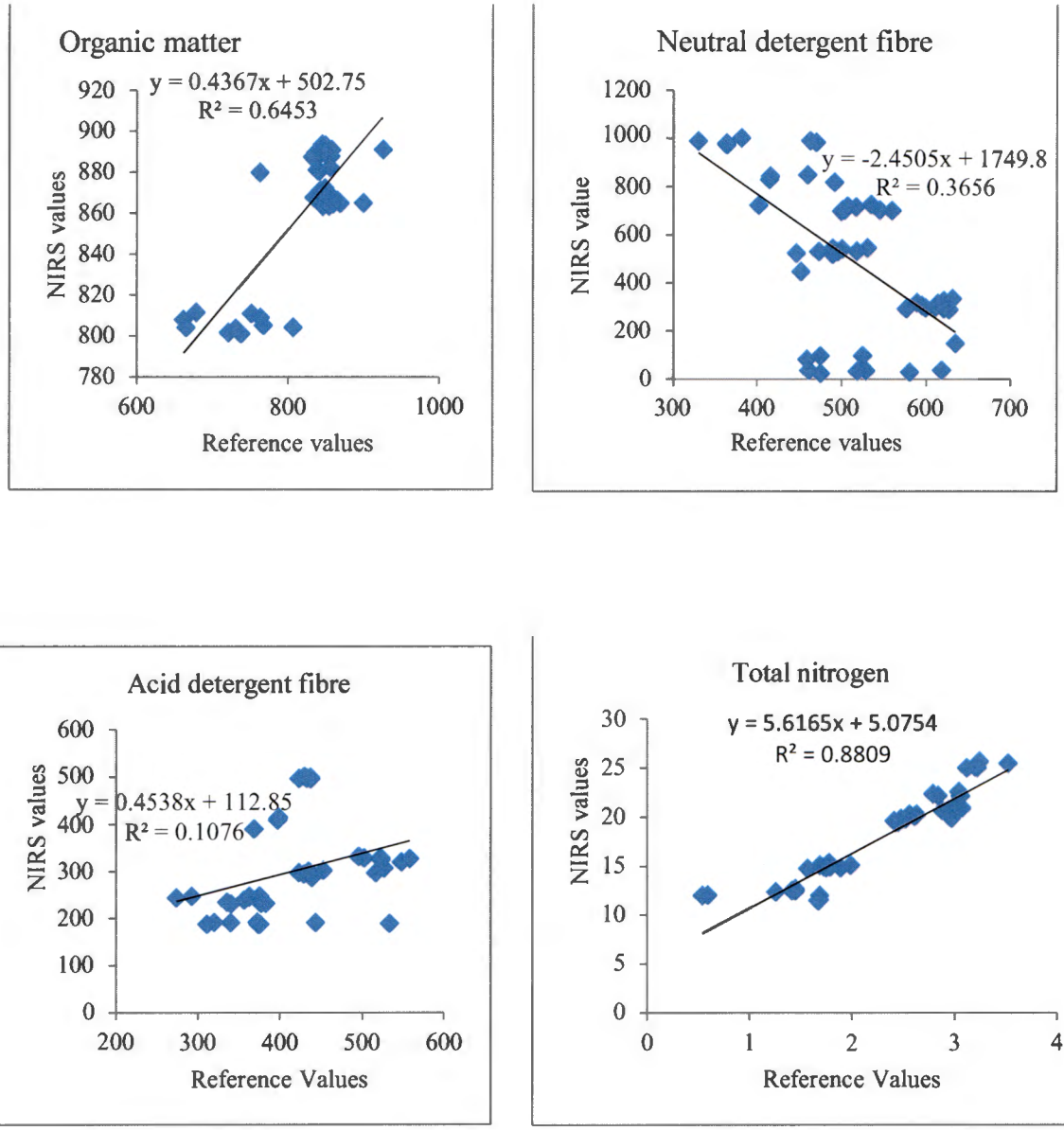

Figure 5.1: Predicted vs reference values in an independent data set

5.5 Discussion

Near infrared reflectance spectroscopy is one of the latest forage and feedstuff analysing technique that has attracted a lot of attention from researchers such as Foley *et al.* (1998). Lang *et al.* (2015) also verified that NIRS has great capability of prediction of chemical composition of plants as most species they scanned were accurately predicted (75–100% correct identifications), and only three had poor predictions (27–60%). Mnisi & Mlambo (2017) reported that all chemical components they tested had R^2 values higher than 50%, whereas in some samples recorded over 90%, showing that NIRS has great ability of predicting nutritional parameters accurately.

5.5.1 Chemical composition and ruminal degradability prediction ability of NIRS

The NIRS prediction models for the browse tree leaves of the current study were very high. The results are in line with calibration models developed by Mnisi & Mlambo (2017), who obtained R^2 values of 0.98 and 0.991 for N and ADF, respectively, while for OM a moderate R^2 value (0.548) was reported. Andrés *et al.* (2005) reported R^2 values greater than 0.95 for CP and NDF. On the other hand Cozzolino & Labandera (2000) reported that for CP, the NIRS calibration statistics yielded R^2 and SECV (in parentheses) values of 0.96 (7.7), ADF 0.98 (16.5) and NDF, 0.96 (34.3) for whole maize plant.

The calibration models for SPh in the current study explained only 40% of the variation, a result that is comparable to the 42% reported by Mnisi & Mlambo (2017) for the same phenolics fraction, showing that NIRS has poor predictive capability for SPh for browse plant leaves. The prediction for condensed tannins had higher R^2 value (0.92) than the 0.72 in *Salix caroliniana* leaves reported by Lavin *et al.* (2015). The prediction for SPh and CTs is not what was anticipated; since CTs are a subset of SPh thus the expectation is that SPh should be predicted with higher

accuracy than CTs. This was the case even in the report by Mnisi & Mlambo (2017), which could be an indication that NIRS is poor on predicting plant secondary metabolites especially when quantified colorimetrically rather than gravimetrically. They also reported prediction accuracy greater than 81.5% for BSN of browse leaves, which was lower than the 88% obtained in the current study.

The calibration models for immediately degradable fraction (50%) on the current study was higher than 12.5% for barley straw reported by Mathison *et al.* (1999) and degradability rate of the dry matter (c) had R^2 values below 0.5 while for degradable part of the insoluble fraction of dry matter (b) (69%) higher than 48.2% by Mathison *et al.* (1999). predicted forage DMED values and reported reasonable accuracy ($R^2CV = 0.75$, RPD = 2.07) Spectral data explained 45% of the variation in effective degradability which was lower than 75% predicted by Belanche *et al.* (2014) of browse plants while Andres *et al.* (2005) presented high predictions (89%) for meadow herbages they analysed. Belanche *et al.* (2014) also reported 75% for potential DMD while prediction for the current study was poor (21%), which was in the same range as 18% predicted by Mathison *et al.* (1999). The differences could be due to the low degree of precision of the *in vitro* technique at early incubation times because of differences in the density and compositing of the rumen liquid between animals and the possibility of physical leakage of the feed from the bags (Belanche *et al.,* 2014). The prediction of immediately degradable N fraction (a) was high (82%), though lower than >92% reported by Hoffman *et al.* (1999) predicted in situ CP fractions. They predicted higher degradation rate was 87% than 14% for the current study. Foskolos *et al.* (2015) predicted 77% for the effective degradability of CP while the current study reported lower R^2 of 60%.

5.5.2 Validation of NIRS predicted values

Validation of calibration models was done using reference values from an independent set of browse leaves. The regression plots reveals that total N had the relatively high R^2 value (0.88) and the least SEP (16.34) revealing excellent capability of NIRS to predict N content in browse plant leaves from different growth environments. The obtained results are in line with Mnisi & Mlambo (2017) who reported a high R^2 value (0.82) and a low SEP (18.19) for total N, showing a good accuracy in the prediction for N content of browse plant leaves. The high accuracy of estimation indicates that NIRS is a suitable method to predict crude protein content in a variety of browse plant leaves. Lavin *et al.* (2015) also used NIRS to assess the nutritionalcomposition of willow (*Salix caroliniana*) and obtained good prediction statistics for CP (R^2 = 0.99, SECV = 0.95), and NDF (R^2 = 0.95, SECV = 2.79) in plant leaves. The model for organic matter prediction had a good R^2 value (0.65). However, the low R^2 value obtained for validated data of NDF (0.37) in the independent sample of browse leaves was, in fact, higher than the 0.20 reported by Mnisi & Mlambo (2017), though the accuracy of prediction is still poor in both cases. On the other hand, Corson *et al.* (1999) reported outstanding prediction statistics for CP (R^2 = 0.99, SECV = 0.95), and NDF (R^2 = 0.95, SECV = 2.79) on the Brassica (kale, rape, chow mollier, turnips, swedes) they analyzed. Spectral data explained 81% of the variation in N degradability at 12 hours, 82% at 24 h and 34% at 36 hours, while Mnisi & Mlambo (2017), reported 42.8% and 77.55% at 24 and 36 hours, respectively.

Corson (1999) indicated that there are various factors that could lead to differences in predictions (R^2) of constituents forage types. They include the range of values within a forage type and interference due to other constituents, such as the influence of the presence of volatile fatty acids on the dry matter determination because of losses during drying, and this affects estimates of composition (Corson, 1999). Other sources of variations could be sample size, as they dealt with

60 samples while in the current study 100 samples were used. Foley *et al.* (1998) indicated that the precision of NIRS-based prediction is greatly affected by the spectral data size and the quality of wet chemistry data used to develop statistical equations. Norris (2009) emphasized that the accuracy of predictions can be attained through the use of larger set of samples in final models. The variation on CP, NDF and ADF content predicted by different authors could also be due to genetic differences of plants, as they contain different levels of these parameters, which can also be influenced by abiotic factors such as location. These factors may lead to varying levels of chemical components of different plant parts, which affect bonding of the chemicals. Corson (1999) indicated that the diffuse reflection have information that detects chemical bonds in the sample, such bonds include CH, -OH, -NH and -SH and the spectra depend on the type and number of such bonds in the sample analysed (Foley *et al.*, 1998). Corson *et al.* (1999) also revealed that the results obtained from NIRS are greatly affected by calibration data derived from laboratory analysis. Landau *et al.* (2006) stated that factors such as laboratory errors during analyses can lead to poor validation, especially that the calibration samples and validation samples were analysed in different laboratories at different times and by different people.

5.6 Conclusions

It can be concluded that the NIRS model for total N content may be utilized for prediction of nitrogen content of browse leaves, as validation had high R^2 value (> 80%) that accompanied calibration models. This technique also had a good predictive ability for organic matter. The NIRS models had low prediction accuracy for NDF and ADF content of these browse tree leaves. The poor prediction could be due to inaccuracy or errors that occurred during laboratory analysis and the small sample size, which could have limited the prediction accuracy of the NIRS models. Some parameters such as nitrogen solubility, dry matter degradability, and nitrogen degradability were not validated due to shortage of external reference samples. Therefore, more work needs to be done on a larger set of samples to improve accuracy of calibration and validation.

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6 CHAPTER SIX: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 Discussion

Browse trees form an integral part of natural source of feed for livestock in Eastern Cape province and plays a crucial role as a source of quality feed. They are able to maintain high nutritional value as their leaves remain green for a longer part of the dry season and serve as supplementary feed when the quality of grass has declined. The area is predominantly savanna and comprising of subtropical thicket vegetation dominated by deciduous woody shrubs shorter than 1.5 m although the woody layer can reach up to 5 m (Scogings *et al.,* 1996). Some of the browse plants in Soxhada communal grazing area include *Maytenus capitata, Olea africana, Coddia rudis, Carissa macrocarpa, Rhus refracta, Ziziphus mucronata, Boscia oliedes, Grewia robusta, Phyllanthus vessucosus* and *Ehretia rigida.* Since these browse trees are exposed to various biotic and environmental challenges, it is anticipated that their nutritional value is bound to change as they respond to such challenges. The area is predominantly used by livestock, comprising of cattle, goats and sheep and a few wildlife species such as kudu. For efficient utilization of scarce feed resources, one needs to know and understand their nutritional value hence they were sampled at various harvesting heights for nutritional analysis.

Browse tree leaves had moderate to high levels of N in agreement with most researchers who have documented that they contain higher N than most grasses and are a major source of protein during dry seasons (Al shafei & Nour, 2016). The results reveal that an interaction between plant species and harvesting height had a significant influence on N content of browse leaves. Height on its own did not have an effect on N content, which could have been due to growth pattern of the trees, others are bushy hence new nutritious shoots grew within browsable height. It is also

worth noting that all browse leaves had higher N than minimum (80 g/kg N) required by ruminants.

Fibre, especially, NDF, ADF, ADL and cellulose play vital roles in ruminant nutrition, as a source of energy for ruminal microbes, maintenance of normal rumen pH and rumen motility. The interaction between tree species and harvesting height did not affect accumulation of fibre, rather the only source of significant variation was due to tree species thus genetic composition was critical. The concentration of fibre in the leaves of these ten browse species was within the range (24–61 %) reported by other authors and generally for forages used in ruminant feeding (Meissner *et al.*, 1991) and above the minimum requirerd for proper rumen function.

The interaction between browse species and harvesting height did not influence concentration of all macro minerals in the browse leaves. The concentration of different macro-minerals was significantly different between tree species. This difference could have been due to differing genetic ability to absorb minerals from the soil since all these trees grew in the same area, under similar growing conditions.

Micro minerals of these ten browse leaves were also determined as they are also vital in animal health and production although the body needs them in minute quantities. Interaction between tree species and harvesting height did not affect micro mineral concentration, rather species did. All the browse samples analyzed had sufficient Fe level to meet requirements for dairy and beef cattle (50 mg/kg DM) though they were below the level reported by Ogunbosoye *et al.* (2015).

Tree species and their interaction with harvesting height had an influence on soluble phenolic content while height did not influence phenolic accumulation. It was expected that harvesting

height would influence distribution of phenols, as plants would channel their defense substances to young, nutritious plant parts which normally happen at the top part of the tree branches, but this was not the case. Browse plants were growing under the same communal area and exposed to similar environmental factors but their phenolic and tannin content varied, which could indicate genetic variation in biosynthesis of secondary plant compounds. Condensed tannins form complexes with carbohydrates and protein making them inaccessible to rumen microbes. The tannins- protein complex may be hydrolyzed under acidic conditions in the small intestines. This property necessitates determination of rumen degradable and undegradable protein in browse plants, so as to know and provide necessary supplements to meet nitrogen requirement of rumen microbes and animal.

One of the procedures used as a proxy for rumen degradable protein is buffer solubility of nitrogen by Licitra *et al.* (1999) but modified by Cudjoe & Mlambo (2014). Cudjoe & Mlambo (2014) indicated that forages with high levels of solubility index are likely to be more degradable in rumen. In the current study, *B. oleoides* (54.64%) at non-browsable height had the highest solubility index, which implies that it can be more degradable in the rumen and can be used to supplement livestock fed on poor-quality diets, which are common during the dry season. At the browsable height (<1.5 m), leaves of *E. rigida, O. africana, C. rudis* and *G. robusta* had the highest insoluble nitrogen, indicating that most of the protein is rumen undegradable, which could be due to tannin content and are suitable for high producing livestock.

The leaves were also analyzed for *in vitro* dry matter and nitrogen degradability. It was established that IVDMD of leaves harvested from both browsable and non-browsable heights was generally low. This could have been due to high fibre content of the leaves, as they contained NDF, which was above 35% and moderate to high condensed tannins which previous researchers established

that they are complex and reduce degradability of the feed. *Boscia oleoides* had the highest IVND and solubility index followed by *Z. mucronata*, *C. mucrocarpa, R. refracta* and *P. vessucosus* and it can be suggested that the leaves of these browse trees can be used as supplement to meet microbial N requirement during dry season, when the quality of pasture is poor and fibrous. *Boscia oleoides* also had the highest immediate degradable 'a' fraction of N of leaves harvested from both heights. Leaves of *P. vessucosus, M. capitata*, *G. robusta* and *C. rudis* harvested from browsable height had higher 'a' fraction than those from non- browsable level, indicating that leaves from browsable height emphasizing their potential crucial use as crude protein supplement for livestock fed poor fibrous diets. Promkot *et al.* (2007) reported low effective CP degradability of cassava hay (47.5%) and indicated that it can be due to high level of rumen bypass protein.

Harvesting height influenced IVDMD and IVND, but did not affect solubility index. The leaves harvested from browsable height had higher IVDMD and IVND than those harvested at non-browsable height, suggesting that they can provide N and energy from fibre to support rumen microbiota than those from non-browsable height. Other browse plants such as *E. rigida* can be used to supplement microbial protein in high producing livestock, as their IVND and solubility indices were low, indicating that their protein is rumen bypass hydrolysable in small intestine. It can also be concluded that solubility index is a poor predictor of *in vitro* ruminal N degradability for all browse leaves except for *P. vessucosus.*

The NIRS prediction models for the browse tree leaves of the current study were very accurate as shown by the high R^2 values which ranged from 0.92 (CTs) to N (0.98), BSN (88%) while SPh (0.40) was the least accurately predicted parameter. Despite that NIRS had poor predictive capability for SPh for browse leaves, the predictability for condensed tannins was high with an R^2

value of 0.90, which was also observed by Mnisi & Mlambo (2017). Low precision of SPh could be an indication that NIRS is poor on predicting plant secondary metabolites or phenolics especially when quantified colorimetrically rather than gravimetrically. Spectral data explained moderate (50%) variation in immediately degradable fraction and degradability rate of the dry matter (c) while for the (b) fraction prediction accuracy (69%) was high. Validation of calibration models using reference values from external, independent set of browse leaves was carried out only on some parameters due to shortage of independent reference values. Poor validation results can occur due to laboratory error during analyses, especially that the calibration and validation samples were analyzed in different laboratories at different times and by different people (Landau *et al.*, 2006).

6.2 Conclusions

The study has shown that the nutritional value of browse plant leaves can be affected by both browse species and harvesting height. Browse plants leaves harvested at browsable height that contained moderate to high N and CTs and macro-minerals included *Ehretia rigida*, *Boscia oleoides*, *Olea africana, Grewia robusta* and *Ziziphus mucronata*. They contain moderate to high level of protein, which was above ruminal minimum requirements and can serve a crucial purpose as a supplement during long dry periods. The different leaves contained high concentration of some macro and micro-minerals although it is advisable to supplement minerals such as zinc and copper, which were below nutritional requirement of animals. All browse tree leaves contained fibre, which could meet the minimum requirement for proper rumen function, maintain normal rumen pH and motility and provide enough energy for ruminal fermentation of feed. Interaction between tree species and harvesting height influenced ash, soluble phenolic content and total N.

The IVDMD of leaves harvested from both browsable and non-browsable heights was generally low, which could have been due to the high fibre which most of them had (an NDF above 35% and possibly above moderate condensed tannins). *Boscia oleoides* had the highest IVND and SI followed by *Z. mucronata*, *C. mucrocarpa, R. refracta* and *P. vessucosus* indicating that they can be used as supplements to meet microbial N requirement during the dry season, when the quality of pasture is poor and fibrous. *Boscia oleoides* also had the highest immediate degradable ' a' fraction of N of leaves harvested from both heights. Leaves of *P. vessucosus, M. capitata*, *G. robusta* and *C. rudis* harvested from browsable height had higher 'a' fraction on N than from non-browsable, indicating that leaves from browsable height had high soluble N emphasizing their potential, crucial use as crude protein supplement for livestock fed poor fibrous diets. Harvesting height influenced IVDMD and IVND, but did not affect nitrogen solubility index.

The NIRS technique can suitably be used to predict total N and organic matter of browse plant leaves, as it had high and accurate prediction for total N and good predictive ability for O.M. Validation for prediction of NDF and ADF was poor, which could have been due to inaccuracy or errors that occurred during laboratory analysis and the small sample size, which could have limited the prediction accuracy of the NIRS models. Some parameters such as nitrogen solubility, dry matter degradability, and nitrogen degradability were not validated due to shortage of external reference.

6.3 Recommendations for future research

There is a need for more research on browse plant leaves, because they have the potential of sustaining livestock since they have high nutritional value. One other study that needs to be done is to determine the effects of condensed tannins in these browse tree leaves on ruminal gas production especially quantification of methane gas, which is one of the green house gases. One of the challenges that the world is facing is global warming, which is mainly caused by greenhouse gases. According to Inthapanya *et al.* (2011), agricultural practices contribute significant amounts (10-12% of total global anthropogenic greenhouse) of which 50% is methane (CH_4). Ruminants are a major source of anthropogenic CH_4, contributing about 33% annually (Beauchemin *et al.*, 2007; Buddle *et al.*, 2011). Some studies have revealed that condensed tannins are capable of decreasing methane production by inhibiting methanogen's growth and indirectly by decreasing rumen fiber degradability hence, a reduced H_2 available for CH_4 production (Tavendale *et al.*, 2005). Feeding trials also need to be done to determine the effects of these browse leaves on microbial load in goats and weight gain of goats. More laboratory analysis are required to build a data base for use on calibration and validation of NIRS for future use to predict nutritional value of browse plant leaves, as it has shown advantages over traditional procedures.

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