The application of near infrared reflectance spectroscopy in the nutritional assessment of tree leaves as potential protein supplements for ruminants

A

Thesis

Submitted in Fulfilment of the Requirements for the Degree of Masters of Science in Agriculture in Animal Science

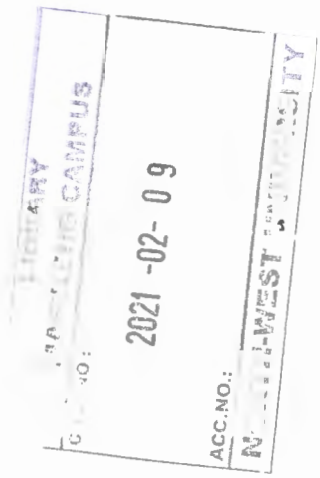
of

The North-West University

by

Mnisi Caven Mguvane

(23257539)



Supervisor

Prof. V. Mlambo

November 2015

Department of Animal Science
Faculty of Agriculture, Science & Technology

DECLARATION

I, Caven Mguvane Mnisi affirm that

i. This is my original research.

ii. The use of information and materials from any other sources has been fully acknowledged.

iii. This thesis is not submitted for any degree or examination at any university other than the North West University.

iv. Reported results were generated by me and not by any other scholar or organisation.

Student: Mnisi Caven Mguvane

Signed: ..... Date: 14/04/2016

Supervisor: PROF. V. MLAMBO

Signed: ..... Date: 14/04/2016

ABSTRACT

This study was executed to calibrate and validate near infrared reflectance spectroscopy (NIRS) for use in predicting chemical composition, buffer nitrogen (N) solubility, *in vitro* ruminal dry matter (DM) and nitrogen (N) degradation and the *in vitro* ruminal fermentation of leaves from *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* tree species harvested from two different growth environments. Section One defines the purpose of the study, while Section Two reviews the nutritional importance of browse leaves as potential sources of nutrients, particularly protein, for optimal animal production. In Section Three, the chemical composition of *A. erioloba*, *A. nilotica* and *Z. mucronata* leaves harvested from Molelwane and Masuthle, which are 40 km apart, was determined. Results from the study showed that growth environment influenced some chemical components but not others. Section Four presents an assessment of buffer solubility of N and *in vitro* ruminal DM and N degradability in tree leaves. Results showed that leaves with high buffer N solubility had high *in vitro* ruminal degradability. However, the presence of secondary plant compounds in the leaves was shown to affect their rumen degradability. Section Five presents an investigation into the *in vitro* ruminal biological activity of tannins present in the tree leaves with the aid of tannin-binding polyethylene glycol (PEG). An automated *in vitro* ruminal gas production technique was used as the tannin bioassay. The PEG inclusion, for all tree species, increased gas production and *in vitro* organic matter degradability; however, it reduced the partitioning factors. In Section Six, the NIRS was calibrated and validated as a rapid technique for the prediction of chemical composition and *in vitro* ruminal degradability of browse leaves. Results showed that NIRS can be a reliable tool to predict total N content because the NIRS model explained more than 80% of variation in total N in an independent sample when externally validated. It was concluded that a

larger number of samples with accurate wet chemistry is required to increase the accuracy of prediction of other chemical components as well as *in vitro* rumen fermentation by NIRS.

Keywords: Chemical composition, Nitrogen solubility, *in vitro* ruminal DM and N degradability, *in vitro* ruminal fermentation, Polyethylene glycol, NIRS, browse leaves

ACKNOWLEDGEMENTS

The financial assistance of the National Research Foundation (DAAD-NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are mine and should not necessarily be attributed to DAAD-NRF. The NWU Top Performers Masters bursary is gratefully acknowledged.

Special thanks to my Supervisor, Prof Victor Mlambo, who made it possible with his everlasting kindness and extraordinary intelligence to execute and finish this study. I thank you Prof for giving me your time, transport to the harvesting sites and all sorts of support and above all great supervision.

To these special people: Dr U Marume, Mr C.S. Gajana, Mr K.E. Ravhuhali, Mr T. Matsogo, Mr A. Tsitsi, and Miss P.S. Mathebula, thank you guys for offering support without even noticing and complaining.

I am indebted to Miss M.S. Tsheole, the Senior Laboratory Technician, in Animal Health Centre, and other support staff who assisted me with mineral analyses. Last but not least, I am grateful to my family for their love and encouragements.

All praises should go up to the loving GOD who makes everything possible. Thank you Father

DEDICATION

I dedicate this thesis to my beloved late brother Sicelo Bruno Thulane Loduka Mnisi.

May his soul rest in eternal peace

"When they ask you how you did it, tell them the God of Mount Zion guided you"

B.E. Lek

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ADF	:	Acid Detergent Fibre
AGRILASA	:	Agri Laboratory Association of Southern Africa
AOAC	:	Association of Official Analytical Chemists
AU	:	Absorbance Units
BINSN	:	Buffer-insoluble nitrogen
BNS	:	Buffer nitrogen solubility
CP	:	Crude Protein
CT	:	Condensed Tannins
DM	:	Dry Matter
HT	:	Hydrolysable tannins
iOMD	:	*in vitro* ruminal organic matter degradability
N	:	Nitrogen
NDF	:	Neutral Detergent Fibre
NDS	:	Neutral detergent solution
NIRS	:	Near Infrared Reflectance Spectroscopy
NRC	:	National Research Council
OM	:	Organic Matter
PCA	:	Principal Component Analysis
PEG	:	Polyethylene Glycol
PF	:	Partitioning Factors
PLS	:	Partial Least Square
SAS	:	Statistical Analysis System
SCT	:	Soluble Condensed Tannins
SECV	:	Standard Error of Cross Validation
SI	:	Solubility Index
SNV	:	Standard Normal Variation
SPh	:	Soluble Phenolics
TAE	:	Tannic Acid Equivalents

1 INTRODUCTION

Ensuring optimal nutrition for ruminants has been a major challenge to many farmers in arid and semi-arid areas of the world due to the increasing cost of commercial feed and fluctuations in quality and quantity of forages. This dissertation continues the search for cheap and affordable feedstuffs as a way to find alternative and nutritious sources of feed that can benefit resource-poor farmers during periods of feed shortages. Southern Africa consists of seven different types of biomes that are a home to a large group of domestic and wild animals (Du Toit *et al.*, 2010). These biomes are natural sources of pastures, which support diverse and dynamic groups of animal and plant species. It is within these biomes that we find *Acacia erioloba*, *Acacia nilotica* and *Ziziphus mucronata* tree species, which have the potential to be cheap sources of green nutritious browse trees that can be used to feed ruminants during the prolonged dry seasons, which last for about eight months (Tefera *et al.*, 2008).

These tree species are widely distributed in the tropical areas of South Africa and they are well adapted to high environmental temperatures and low rainfall regions. Their wide distribution and capacity to withstand harsh environmental conditions make them a valuable fodder source for animal feeding during the dry season, where feed quality and quantity is inadequate. These tree species can be used as strategic supplementary feeding to alleviate feed shortages during winter (Mlambo *et al.*, 2008) because they retain green foliage better than grasses. Bruno-Soares *et al.* (2011) amongst many other scholars, supported the idea that the use of these browse trees can be a strategic approach to provide green forage to herbivorous ruminant animals. During the long dry seasons in the North-West province of South Africa, grass foliage is characterized by low protein and high lignin concentrations. Mature grass foliage, therefore, provides low amounts of nutrients to ruminant and reduces voluntary feed intake, all of which

negatively affect livestock performance (Garcia, 2013). Browse trees can be used to supplement poor quality grass and cereal crop residues commonly consumed by ruminants in semi-arid areas. The importance of these tree species is defined by their ability to provide proteins, energy, minerals and vitamins to ruminants in periods when grasses are deficient in some of these nutrients or when there is abundance of poor quality roughages with limited amount of proteins and other essential nutrients.

While browse trees are widely accepted as potential sources of protein to ruminant animals, they contain antinutritional factors that may affect the nutrition and health of livestock. Of concern is the high concentration of polyphenolic compounds such as tannins in some of the browse products. *Acacia erioloba*, *Acacia nilotica* and *Ziziphus mucronata* tree species are ubiquitous in the semi-arid regions of the North-West province but their utility as sources of protein for ruminants has not been evaluated. The concentration and biological activity of these polyphenolic compounds vary with tree species as well as the growth environment, among other factors. The need to reduce the negative effect of tannins and increase their beneficial contribution to ruminant production is thus of economic importance.

1.1 Justification

The exploitation of browse-trees is important in providing nutritionally adequate feed to ruminants. However, the nutritive value of browse products varies with growth environments, as well as tree species. The utilization of these browse products is also complicated by the presence of plant secondary compounds (phenolics) whose nutritional effect is greatly undefined in the rangelands of the North-West province. To ensure a judicious use of browse leaves in ruminant diets, it is important that their nutrient composition be accurately determined. Traditional nutritional evaluation

procedures are often time-consuming, costly, and pose a challenge to the environment when it comes to waste disposal. With accurate calibration, rapid nutritional evaluation techniques such as the near infrared reflectance spectroscopy (NIRS), have the potential to determine the nutritive value of browse leaves in real-time (Landau *et al.*, 2006). The accurate and rapid nutritional evaluation of these tree species would provide information required to formulate diets that maximise animal performance and health.

1.2 Objectives

The study was designed to investigate the nutritive value of leaves from *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from two different sites and assess the utility of NIRS as a tool for a non-destructive evaluation of the leaves. The following specific objectives guided the study:

1. To determine the chemical composition, buffer-soluble nitrogen, *in vitro* ruminal dry matter and nitrogen degradability, and the *in vitro* ruminal biological activity of tannins of leaves from the *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from two different growth environments.
2. To calibrate and validate the near infrared reflectance spectroscopy (NIRS) for use in predicting chemical composition and *in vitro* ruminal fermentation of leaves from the *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from two different growth environments.

1.3 Hypotheses

This study tested the alternative hypotheses that:

1. Tree species and growth environment cause variation in nutritional parameters as assessed by chemical analysis, buffer nitrogen solubility, *in vitro* ruminal nitrogen degradability and *in vitro* ruminal gas production.
2. The NIRS technique provides spectral variables with nonzero coefficients, which can predict the nutritional value of browse leaves.

1.4 References

Bruno-Soares, A.M., Matos, T.J.S., Cadima, J., 2011. Nutritive value of Cistus salvifolius shrubs for small ruminants. Anim. Feed Sci. Technol. 165, 167–175.

Du Toit, J.T., Kock, R. & Deutsch, J.C., 2010. Wild Rangelands: Conserving wildlife while maintaining livestock in semi-arid ecosystems. Pp.1 - 2.

Garcia, S.N., 2013. Chemical composition, nitrogen buffer solubility and simulated rumen degradability of potential non-conventional protein supplements. A thesis. Pp.: 1 – 95.

Landau, S., Glasser, T. & Dvash, L., 2006. Monitoring nutrition in small ruminants with the aid of near infrared reflectance spectroscopy (NIRS) technology: A review Small Rumin Res. 61, 1 – 11.

Mlambo, V., Mould, F.L., Sikosana, J. L. N., Smith, T. Owen, E. & Mueller-Harvey, I., 2008. Chemical composition and *in vitro* fermentation of tannin-rich tree fruits. Anim. Feed Sci. Technol. 140, 402 - 417.

Tefera, S., Mlambo, V., Dlamini, B.J., Dlamini, A.M., Koralagama, K.D.N. & Mould, F.L., 2008. Chemical composition and *in vitro* ruminal fermentation of common tree forages in the semi-arid rangelands of Swaziland. Anim. Feed Sci. Technol. 142, 99 - 110.

2 LITERATURE REVIEW

2.1 The *Acacia* genus

Acacia erioloba and *A. nilotica* tree species, originate from the genus *Acacia*, which is a large genus of shrubs and trees occurring all over the tropical regions, mostly where temperatures are warm. The largest number of species in this genus is found in Australia where they have in the region of 900 species (van Wyk *et al.*, 2000). The name *Acacia* comes from the 'Greek' word which means thorns or sharp point, since a large number of these species are thorny. *Acacia* belongs to the subfamily *Mimosoideae* from the family *Fabaceae/Leguminosae*. According to van Wyk *et al.* (2000) *Acacia* species are the third largest woody plant family consisting of about 100 tree species in South Africa. The other common member of this family is *Albizia* which is easily distinguished from *Acacia* because their plants have no thorns. Both *Acacia* and *Albizia* are important ecological components throughout the various rangelands areas of the country (Davidson, 1981). This is because they are an important source of proteins for a large number of naturally occurring animal species such as goats, sheep, cattle and other wild animals, especially in the dry seasons.

Acacia erioloba and *A. nilotica* tree species are some of the protected *Acacias* in South Africa in terms of Section 12 of the National Forests Act, 1998 (Act No. 84 of 1998). Under this act, "No person may (a) cut, disturb, damage, destroy or remove any protected tree; or (b) collect, remove, transport, export, purchase, sell, donate or in any other manner acquire or dispose of any protected tree, except under a licence granted by the Minister" (Seymour & Suzanne, 2003). This act does not differentiate between dead and live trees, which reflect that the removal of wood or dead tree is also against the law. If this law can be honoured, sustainable development and veld stability can be maintained and in the process, most rangelands can become productive because of no

deforestation and other disturbances. Rangelands can therefore remain a continuous supplier of nutritious feed to the diverse groups of wildlife and domestic animals, and also resulting in lower feeding costs and increased farming profits.

2.1.1 *Acacia erioloba* tree species

Acacia erioloba is tree number 168 in South Africa from the family *Fabaceae* and is commonly known as camel-thorn (Ecocrop, 2012). This is a multipurpose tree species which is protected in South Africa. They are slow growing and can survive under poor or saline soils and harsh environmental conditions (Brenan, 1983). Their ability to withstand harsh conditions has promoted their wide distribution all over the country although their distribution is limited by freezing conditions in temperate regions. After successful establishment, seedlings can still be vulnerable to harsh conditions such as drought, frost, herbivores and fire, but large matured trees survive these harmful conditions (Seymour & Suzanne, 2003). *Acacia erioloba* ranges from approximately 2 m shrub to 16 m big tree in height and their stem is reddish-brown when young and their bark becomes grey-blackish brown when they are matured (van Wyk & van Wyk, 1997). They have white or brown-like spines that may be about 60 mm long, and their leaves are divided into two parts with 2 to 5 pairs of pinnae per leaf, together with 8 to 18 pairs of leaflets per pinna (Smith, 1999; Brenan, 1983).

According to van Wyk *et al.* (2000), the camel thorn tree bear bright yellow ball-like flowers in the late periods of the winter up until summer season. They begin fruiting at about 10 years of maturity (Coe, 1998) and their fruits, which are of nutritional importance, are variable and range from small and almost cylindrical to typically large, flat, thick and hairy-covered (Seymour & Suzanne, 2003). Their seeds are thick, robust and lens-shaped (Davidson, 1981). According to Smith (1999), they bear fruits that are

semi-woody, but spongy inside. Their pods do not open even when ripe but fall to the ground during winter seasons. These pods are a useful source of fodder for cattle and are highly favoured by wild animals in Africa, especially elephants who chew the pods and disperse the seeds in their dung (Seymour & Suzanne, 2003). *Acacia* tree species have the potential to be used as fodder sources or protein supplements for livestock, and this is based on their moderate to high crude protein value, low acid detergent fibre and tannin content, which increases the intake rate and relative palatability indices (Aganga *et al.*, 2003).

Leistner (1961) suggested that the nutritive value of the *Acacia* pods can be compared with that of lucerne. Seymour & Suzanne (2003) supported this idea by one of their studies conducted in Zimbabwe that found these pods to be higher in protein and acid soluble mineral than the grasses from rangelands. Coe (1998) suggested that the indehiscent pods, in particular, have lower tannins but high protein levels such that they can be considered a valuable food source for livestock. Timberlake *et al.* (1999) also elaborated that pods provide a nutritious browse supplement, and when crushed with the seeds, they can contain between 10 - 20% proteins. They further suggested that some farmers or ranchers harvest the pods and mill them with sulphur (in order to neutralise prussic acid contained within the pods) to supplement cattle feeds in times of drought or feed shortages. This harvesting is done to enable herds to survive during the prolonged periods of the dry season where feed sources are depleted. The nutritive values of camel thorn pods need more investigation in order to recommend their correct utilisation by ruminants.

The browse products from *A. erioloba* tree species should be efficiently utilised to provide livestock with feed that contain high concentrations of proteins and other nutrients. This can help reduce nutrient deficiencies that may lead to metabolic diseases,

which is a major factor that negatively affect ruminant production. According to Ngwa *et al.* (2000), one way of alleviating the problem of feed shortages is through the correct use of forages from tree legumes which can provide a good source of protein, vitamins and minerals to animals during critical dry periods of the year, especially when pastures are deficient both in quantity and quality. Metabolic diseases can cause poor growth rate and lowers birth, weaning and yearling weight and ultimately decreasing productivity in most communal farms and other commercial farming systems in South Africa. *Acacia erioloba* species serve as a good source of the much needed nutrients to both domestic and wild herbivores and some wildlife species (Aganga *et al.*, 1998).

Plate 1: Leaves of the *Acacia erioloba* (ecoport.org)

2.1.2 *Acacia nilotica* tree species

Acacia nilotica commonly known as scented-pod *Acacia* or Babul, is tree number 179 in Africa. This *Acacia* is a multipurpose tree species which originates from Africa, the Arabian Peninsula and the Indian subcontinent (Ecocrop, 2012). It is now commonly found or cultivated within 30° North and 20° South in almost all tropical and subtropical areas of Africa, Asia, Australia and the Caribbean (Fagg & Mugedo, 2005; Orwa *et al.*, 2009; Ecocrop, 2012). From the nine *Acacia nilotica* subspecies, two are

found in the Indian subcontinent, namely *cupressiformis* and *hemispherica*, and six are found in Africa and known as *indica*, *kraussiana*, *leiocarpa*, *nilotica*, *subalata*, *tomentosa*, while *adstringens* occurs in both continents (Ecocrop, 2012). These subspecies are distinguished by their shape and a pubescence of their pods. Babul is medium sized and thorny hence it is from the *Acacia* genus. It is nearly an evergreen tree with a height of about 20 – 25 m but may remain a shrub in poor growing conditions (Fagg & Mugedo, 2005; Orwa *et al.*, 2009; Ecocrop, 2012). Scented-pod *Acacia* have a short trunk which is thick and they are cylindrically covered with a grey bark (Brenan, 1983). According to Fagg & Mugedo (2005), Babul has a crown that may be flattened or rounded, and their root system is influenced by the growing conditions and their subspecies. They develop a deep taproot in dry conditions, and extensive lateral roots in flooded/wet conditions (van Wyk *et al.*, 2000). The leaves are about 5-15 cm long, alternate and compound with 7 to 36 pairs of elliptical (Cook *et al.*, 2005). They consist of grey-green hairy leaflets together with flowers that are sweetly scented and bright to golden yellow in colour (Davidson, 1981). The fruits are linear with flattened and narrow indehiscent pods and they are about 4-22 cm long and 1-2 cm broad (van Wyk & van Wyk, 1997). The colour of the fruits is green when young and dark-brown to grey when fully matured. The pods of Babul contain 8 to 15 elliptical, flattened bean-shaped dark seeds (Cook *et al.*, 2005; Fagg & Mugedo, 2005; Orwa *et al.*, 2009). These fruits are protein sources for livestock during periods when rainfall is low and feed sources are lowest, which means that these fruits can be used to supplement the protein content of most feeds during the dry season. Aganga *et al.* (1998) reported that *A. nilotica* tree species can act as nitrogen-fixing legumes in places where grass is growing and may help improve their nutritive value.

Acacia nilotica pods have a characteristic "necklace" shape with constrictions between the seeds (Brenan, 1983). These seeds pass through the animals' digestive system in large proportions undigested. However, crushing or changing the physical state of the seeds before feeding may help in making the nutrients that are contained by seeds to be available to animals. The tree leaves are browsed by livestock for fodder and can be a fundamental source of nutrients during the dry season when there is an increase of poor quality feeds (Orwa *et al.*, 2009). The fruits can be eaten on the ground or browsed by livestock or they can be harvested and fed to livestock. Orwa *et al.* (2009) reported that *A. nilotica* is a useful fodder source, particularly in dry regions where feed supply is low. The forage management of *A. nilotica* is complex because various parts of the plant are used at different periods of the year for feeding different types of animals.

Plate 2: Leaves of the *Acacia nilotica* (biodiversityofindia.org)

2.2 The *Ziziphus* genus

The *Ziziphus* genus consists of about 140 tree species found in the southern Africa (Aganga & Mosase, 2001). The genus *Ziziphus* is cosmopolitan and is sub-tropically distributed. Amongst the hundred different tree species found in this genus, there is *Ziziphus mucronata* which belongs to the *Rhamnaceae* family existing in the bushveld, woodland and grassland hills or along river banks. Most trees from this genus that are drought-tolerant and can grow well during lean periods (Osuga *et al.*, 2007). Trees from this genus can be used by human beings for numerous reasons and are of economic importance, especially the *Z. mauritiana* together with the *Z. jujuba* because they are considered as fruit-trees in China and India, where they had been grown for many years (Maier *et al.*, 2006).

2.2.1 *Ziziphus mucronata* tree species

Ziziphus mucronata is commonly known as buffalo thorn mainly because its thorns reflect the shape of buffalos' horns. This tree species is widely distributed across southern Africa and is found in arid and semi-arid regions that are dominated by thorny vegetation at an altitude of 2000 m above sea level (Ecocorp, 2014). The buffalo thorn has the ability to withstand drought and frost environmental conditions and can grow from various soil types. According to Hassen *et al.* (2009), *Z. mucronata* is a valuable fodder tree found in the drier regions of Africa and is tolerant to climatic variability. *Ziziphus mucronata* is a small to medium sized tree species which ranges from 5 – 10 m high, but it can sometimes go beyond 10 m. This tree species has an irregular crown and has drooping branches that are armed with pairs of sharp thorns ranging from 0.7 – 2 cm on each node (Heuzé & Tran, 2014). Buffalo thorn has a short trunk that is about 40 cm in diameter, branching near the base with a bark that is grey–brown in colour. Their leaves are simple and alternate with different sizes that are about 3 – 9 cm long and 2 –

5 cm broad (Heuzé & Tran, 2014). The colour of their leaves is glossy green on the upper side and pale-green on the lower side/below. This species has small flowers (4 mm) that are green to yellow and borne clusters. Flowering is strongly influenced by rainfall but most occurring around October to the late summer. *Ziziphus mucronata* bears many fruits that are usually observed with branches bending under fruit weight. Their fruits are range from 10 – 20 mm and resemble globuse and glossy drupes that changes colour from green to dark brown as they ripen late in the summer/winter (Heuzé & Tran, 2014). Orwa *et al.* (2009) stated that their exocarp is hard and shiny while their mesocarp is floury and nutritious. *Ziziphus mucronata* is a multipurpose tree that is of nutritional and cultural importance in Southern Africa, though its importance varies from one traditional group to another (Mazibuko, 2007). Rothaugue *et al.* (2003) reported that the leaves of buffalo thorn are nutritious and the fruits are edible to both human beings and ruminants. The fruits of *Z. mucronata* form part of people and animals' diet while the leaves are valued for their foliage for browsers (Aganga & Mosase, 2001).

The buffalo thorn's fruits are edible and nutritious but they are not very tasty; during the dry season, they can be consumed when they are fresh and can be made into a ration and the fruit flesh can be mixed with water and fermented to prepare beer by human beings (Roodt, 1998). The leaves are not palatable to human beings but can be nutritious to animals. Their high tannin content can be used in tanning leather tanks. Orwa *et al.* (2009) stated that both leaves and fruits from *Ziziphus mucronata* can be a forage source for livestock. The moderate protein value (14 %), fibre content and high mineral content have made this tree species to be of great importance for ruminant production. *Ziziphus mucronata* is ranked amongst the other top trees that provide nutritious forage which is palatable to livestock especially goats (Ondiek *et al.*, 2010).

Plate 3: Leaves of the *Ziziphus mucronata* (zimbabweflora.co.zw)

2.3 The importance of the browse trees

Emerging farmers heavily rely on rangelands to feed their livestock, this is always an advantage in extensive farming systems where most farmers do not have enough funds to purchase feed supplements for their livestock. Domesticated animals can depend on browse trees available for feeding during the winter seasons when there is little or no rainfall. Browsers such as goats have easy access to dry and ripe leaves and fruits as they fall from the trees to the ground at the start of the dry season around June and they have the ability to utilise these browse products efficiently (Mlambo *et al.*, 2008). The dry season is characterised by extreme temperatures, increased feed shortages; most rangelands are dry and degraded by factors such as droughts, erosion, deforestation and low rainfall, which eventually result in reduced productivity of livestock. The reduction in productivity is characterised by delayed puberty, anoestrus, low conception and fertility rate, increased metabolic diseases, low growth rate and high mortality rate in

livestock (Mlambo *et al.*, 2008). *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* browse trees can be exploited to help provide leaves and fruits to supplement the protein requirement of ruminant animals and improve their nutritional status. Both leaves and fruits from these tree species play an important role in animal nutrition because they serve as potential sources of nutrients in the dry season. Collection of fruits can be easily practised by farmers and preserved for later use as protein supplements (Mlambo *et al.*, 2008), while leaves can be easily accessed by animals and utilized at any stage of growth because they have low lignin content than fruits especially those of *A. erioloba* tree species.

Poorly managed pastures lead to nutritional deficiencies and low voluntary intake which therefore reduce animal production (Cudjoe & Mlambo, 2014). Nutritional imbalances are major factors which negatively affect livestock production as they increase the prevalence and the large scale occurrence of metabolic disorders. Ngwa *et al.* (2000) suggested that low quality pastures, the seasonal nature of forage supply, and low intake together with digestibility of forages are some of the factors that reduce productivity of ruminants in Africa. Mlambo *et al.* (2008) reported that insufficient quantity and poor feed quality result in decreased livestock productivity in tropical countries. According to Tefera *et al.* (2008), these green nutritious browse trees play a major role in supporting year-long productivity of livestock in the arid and semi-arid regions of Southern Africa. This is because these browse trees remain green and nutritious during the dry periods and can be preserved and fed to most livestock as supplementary feeds during periods of feed shortages or when protein-deficient pastures are prevalent. Currently, arable land is used for growing fodder while natural pastures are restricted to areas that are of good quality for farming, therefore the need to find

alternative sources of feed to provide for livestock animals is essential to maintain production (Kibria *et al.*, 1994).

According to Mlambo *et al.* (2008), browse products can be utilised as a potential source of protein, but more information on their nutritive value needs to be investigated and made available if their use is to be improved and increased. The nutritive value of these browse trees has always been a problem to investigate because it is largely unknown. However, the exploitation of these browse-trees specifically *Acacia erioloba*, *A. nilotica* and *Z. mucronata* is important in providing adequate nutritious feed to livestock during the dry season. These tree species can withstand dry periods and harsh environmental conditions and can grow well in poor soil conditions. It would then be beneficial to evaluate the nutritive value of these trees to determine their potential as protein supplements to ruminant animals. Evaluation of the nutritional parameters of these trees gives more information on their nutritive values. Terblance *et al.* (1967) reported threats caused by fruits of *A. nilotica* when consumed in large quantities by goats, hence, an investigation of the nutritive value of these browse products would help guide farmers to provide correct quantities of the browse products to animals in a specific period of time.

2.4 Browse products as gap-fillers in the feed calendar

The effects of veld fires, bush encroachment, deforestation and climatic factors have largely affected the distribution and growth of *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species. According to Mlambo *et al.* (2009) droughts and low rainfall are the sole reasons for the low supply of high quality feed in semi-arid regions and they are causing a constant decline in livestock productivity on smallholder communal farms. These tree species can serve as supplementary feeds to ruminants especially goats and

thus reducing the cost of buying expensive feed supplements. The insufficiency of nutrient-rich feed materials had led to an increase in metabolic diseases because fewer nutrients were consumed than required. Tefera *et al.* (2008) suggested that a total removal of woody plants would increase the nutrient fluctuations of livestock mostly in regions where supplementation is not common. A provision of adequate amount of leaf-meal from these tree species should be maintained in order to maximise animal production. According to Mlambo *et al.* (2008), leaves from these trees could be used as a protein source for animals that are feeding on poor quality roughage with increased level of lignin content predominating later in the dry season. But according to Terblance *et al.* (1967), feeding large quantities of fruits from *A. nilotica* more frequently may result in animals developing haemorrhage lesions in their digestive tracts, causing death of the animals, more especially goats due to their natural ability to feed on different types of plant species such as browse trees and grasses. The mobile upper lip of goats enables them to browse a variety of plants and to obtain feed material that is rich in proteins and other nutrients. They are also capable of breaking the hard-covered fruits of *A. erioloba* to gain access to the nutrient content of the fruits and this is one of the reasons why goats can survive the dry period and maintain optimum production under harsh conditions.

2.5 Chemical composition and nutritional value of browse products

The determination of nutrient content, which is the concentration of certain chemical constituents of plant material, is important. *Acacia nilotica*, *A. erioloba* and *Z. mucronata* supply most proteins from their leaves during the dry season where protein feed sources are limiting, although the nutritive value is unknown. Most tree fruits were reported to contain up to 200 g/kg crude protein (Tanner *et al.*, 1990). The seeds from these tree species are hard-covered which reduces their digestibility as they pass

through the animals' digestive system because microbes cannot fully break them, therefore, crushing of these seeds before giving them to animals would be helpful in availing the protein and other nutrients contained by the seeds. The use of these trees by most farmers as feed supplements is limited because they could be poisonous towards livestock. According to Makkar (2003), the anti-nutritional factors are as a result of stress experienced by plants. Plants become tanniniferous due to the combination and amount of tannins they produce, which eventually lower their palatability. However, the phenolics present in *A. nilotica* negatively affect ruminant productivity although their utilisation can be improved by ruminal adaptations (Mlambo *et al.*, 2008).

Aganga *et al.* (2003) reported that the crude protein content of *A. erioloba* was lower than that of *A. nilotica*. Despite the differences amongst the proximate components, these tree species remain a good source of protein supplement for ruminants grazing poor quality roughages. *Ziziphus mucronata* had a tannin content of about 12 – 15%, which can play a vital role in treating dysentery (Ellis, 2003). Buffalo thorn tree species also have a protein content of about 10 – 20% per dry matter (DM) basis and a moderate acid detergent fibre (ADF) content ranging from 17 – 23% per DM basis (Njidda & Olatunji, 2012). This tree species is considered to be a potential protein supplement in poor quality forages and their macro-mineral contents are higher than those required by cattle (Njidda & Olatunji, 2012). Poor quality feeds are defined as those that have a crude protein (CP) value of less than 80 g/kg (Leng, 1990). Aganga & Mosase (2001) have stated that the seeds of *Ziziphus mucronata* have a protein content of 7.1% per DM basis and low tannin content, and can therefore be considered a potential source of nutrients for grazing animals. Foliage from *Ziziphus mucronata* was reported to have medium to high *in vitro* DM digestibility ranging from 55 – 75% (Njidda & Nasiru, 2010). *Acacia nilotica* was reported to have higher tannin content

than *Z. mucronata* (van Hoven & Furstenburg, 1992). The utilisation of browse products can be limited by the high lignin content and the presence of antinutritional factors, which may be toxic to ruminant animals (Njidda, 2010), hence, the need to investigate their nutritive value. Tables 2.1 and 2.2 show the reported variations amongst chemical composition of leaves and fruits from *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species.

Table 2.1: Chemical composition of leaves of the three different tree species (g/kg DM).

Tree species	Chemical composition				References
	NDF	ADF	ADL	CP	
Acacia erioloba	-	-	-	-	
Acacia nilotica	216	143	-	169	Nsalhai *et al.* (2011)
Ziziphus mucronata	337.3	274.7	85.3	188	Hassen *et al.* (2009)

Table 2.2: Chemical composition of pods of three different tree species (g/kg DM).

Tree species	Chemical composition			References
	NDF	ADF	CP	
Acacia erioloba	184.0	113.0	176.4	Aganga *et al.*, (2003)
Acacia nilotica	224.7	180.8	149.1	Ngwa *et al.*, (2000)
Ziziphus mucronata	546	419	70.8	Aganga & Mosase (2001)

The mineral content in feed materials also forms part of the chemical composition which a feed sample can be analysed for. Table 2.3 and Table 2.4 adapted from CSIRO (2007) shows the dietary mineral requirements of ruminant animals that when given to animals can maintain growth, production and reproduction.

Table 2.3: Macromineral for ruminant dietary requirements (g/kg DM).

Macrominerals	Cattle	Sheep and goats
Calcium (Ca)	2.0–11.0	1.4–7.0
Phosphorus (P)	1.0–3.8	0.9–3.0
Chlorine (Cl)	0.7–2.4	0.3–1.0
Magnesium (Mg)	1.3–2.2	0.9–1.2
Sodium (Na)	0.8–1.2	0.7–1.0
Sulphur (S)	2.0	1.5

Adapted from CSIRO (2007)

Table 2.4: Microminerals for ruminant dietary requirements (mg/kg DM).

Microminerals	Cattle	Sheep and goats
Cobalt (Co)	0.08 - 0.15	0.07 - 0.15
Copper (Cu)	4 – 14	4 -14
Iodine (I)	0.5	0.5
Iron (Fe)	40	40
Manganese (Mn)	20 – 25	20 – 25
Zinc (Zn)	9 - 20	9 – 20

Adapted from CSIRO (2007)

2.6 Digestive system of ruminants

Ruminants are defined as herbivorous animals that consist of four stomach chambers and can regurgitate their cud. The process of regurgitation takes place in order to further the breakdown feed particles, which were not finely digested, into smaller absorbable particles that can be absorbed by the animals. Cattle, sheep and goats are the main four-chambered domestic animals that can undergo rumination, hence they are called ruminants. The digestive system of ruminants starts from the mouth, tongue, salivary glands, oesophagus, pancreas, gall bladder, the four chambers, the small intestines (duodenum, jejunum, ileum) and large intestines (cecum, colon and rectum). The four chambers that are found in these ruminants include the reticulum, rumen, omasum and abomasum, each playing a very significant role in the digestion and absorption of feed particles. According to Cudjoe & Mlambo (2014), the digestive system of these animals is formed in a way that allows them to efficiently utilise fibrous diets and roughages. Ruminants like any other animals use their lips and tongues found in their mouths to grab and ingest feed. Mastication takes place in the mouth and with the aid of the salivary glands, saliva is secreted to enhance digestion and the channelling of feed particles to the gastro-intestinal tracts. The oesophagus is a muscular tube responsible for the movement of consumed feed to the digestive chambers through a process called peristalsis (Russell *et al.*, 1979). The reticulum plays a peculiar role in trapping foreign objects and channelling feed consumed by the animal into the rumen, while the omasum acts as a sieve, restricting improperly digested feed particles to pass through to the abomasum, hence the regurgitation/rumination process. The abomasum is defined as the true glandular stomach, which is responsible for the absorption of nutrients. The rumen is the largest chamber occupying approximately 75% of the abdominal cavity (Russell

et al., 1979), and is responsible for the breaking down and fermentation of feed particles into absorbable substrates for the host animal.

2.6.1 The role of the rumen

The largest chamber, known as the rumen, physiologically develops as new-born calves are fed on fibrous feed materials and in the process it becomes inoculated with microorganisms. Ruminants have evolved a special digestive system that is microbially fermentative, which enables them to break down fibrous diets. A symbiotic relationship between the host animals and the microbes is formed in the rumen whereby the two positively provide each other, for example, a synthesis of microbial protein from consumed feed and the breaking down of roughages. The rumen wall is lined with rumen papillae which play a role in absorbing volatile fatty acids synthesized from carbohydrates in the rumen. The microbial population includes bacteria, protozoa and fungi and have full potential in breaking down and fermenting feeds into absorbable fractions of volatile fatty acids such as the acetic, butyric and propionic acids (Cudjoe & Mlambo, 2014). According to van Soest (1987), the volatile fatty acids are energy sources for the ruminants. The rumen acts as fermentation vat where carbohydrates are synthesised and broken down from grasses, protein degraded from non-protein nitrogen, and also produces vitamins such as K and B complexes. The microbial population in the rumen can efficiently synthesize feed substrates into microbial proteins that are available for the host animal post-ruminally. In the rumen, feed is in three phases and these are as follows, the lower liquid phase consisting of finer particles, the drier middle layer consisting of coarser solid material, and the upper layer consisting of fermentation gases, such as carbon dioxide and methane and they are released through belching and eructation. Microorganisms can thrive well in an optimal rumen pH of 6.5 – 6.8 (Russell *et al.*, 1979). This microbial population plays a positive significant role in

maintaining the rumen temperature at 38 – 42°C by producing heat through fermentation.

2.6.2 Feed quality for ruminants

Nutrition is a major aspect which affects livestock production, reproduction and growth. For ruminants to become more productive, they must have a balanced ration consisting of all the essential nutrients necessary for maintenance, growth, reproduction and production. Feed quality could mean a daily supply of feed with all essential ingredients without any imbalances or deficiencies. Improper feeding or rather imbalanced diets may lead to metabolic diseases which can negatively affect livestock production. Ruminants normally feed on natural pastures as their major sources of feed, although, feed problems are encountered mostly during lean periods when pastures are dry and have increased levels of lignin with depressing content of protein. Lignin is defined as the indigestible polyphenolic polymer that physically and chemically combines with cell wall contents (Boudet, 1998). The quality of feed also declines during dry seasons and is reflected by a reduced voluntary feed intake, low nutritional value and poor animal performance (Amigot *et al.*, 2005). Feed that is of good quality should provide adequate amounts of protein, minerals, vitamins, and energy and they should have desired levels of phenolic contents with very low lignin content. Quality feed would increase voluntary feed intake, average daily gains and productivity.

2.6.3 Protein quality for ruminants

Ruminants require proteins for growth and development, production and reproduction. Proteins are defined as long and complex organic compounds that are formed when amino acids are combined with each other into polymers. Dietary proteins are hydrolysed to peptides and amino acids by rumen microbes. Some of the proteins from

the feed are converted to microbial protein through the process of ruminal fermentation. Proteins undergo proteolysis which is the splitting of proteins by hydrolysis of the peptide bonds with the ultimate formation of smaller polypeptides. Such proteins are regarded as rumen degradable proteins. Dietary proteins and microbial proteins are the two major sources of protein for ruminant animals. According to Cudjoe & Mlambo (2014), proteins that are degraded in the rumen provide the potential nutritive value of the protein in any feed.

Protein quality can be defined as the ratio of rumen degradable protein to rumen undegradable protein. The ability of a protein to provide all the essential amino acids defines its quality (Cudjoe & Mlambo, 2014). A protein is said to be undegradable if it passes through the rumen unchanged or undigested and reaches the small intestines where they are digested and absorbed. The amount of protein that passes the rumen to the lower tracts for degradation is known as the by-pass protein (Licitra *et al.*, 1996). By-pass proteins are important for high levels of production in a dairy set-up because the animal receives adequate amino acids directly in the small intestines for the production of milk. The supply of essential amino acids from the microbial and dietary proteins is often inadequate for high producing animals, for example, dairy cows and ewes that produce gallons of milk per day require a constant supply of protein supplements. By-pass protein shortens the amount of proteins that are degraded in the rumen by the rumen microbial population because proteins are quickly channelled and deposited into the small intestines. Nitrogen degradation from feed substrates in the rumen strongly depends on the type of feed provided to the ruminant animal. Figure 2.1 below indicates the fate of nitrogen in the rumen from two sets of protein sources – dietary protein and non-protein nitrogen (NPN), which are degraded to form the microbial protein that is utilised by the rumen microbial population.

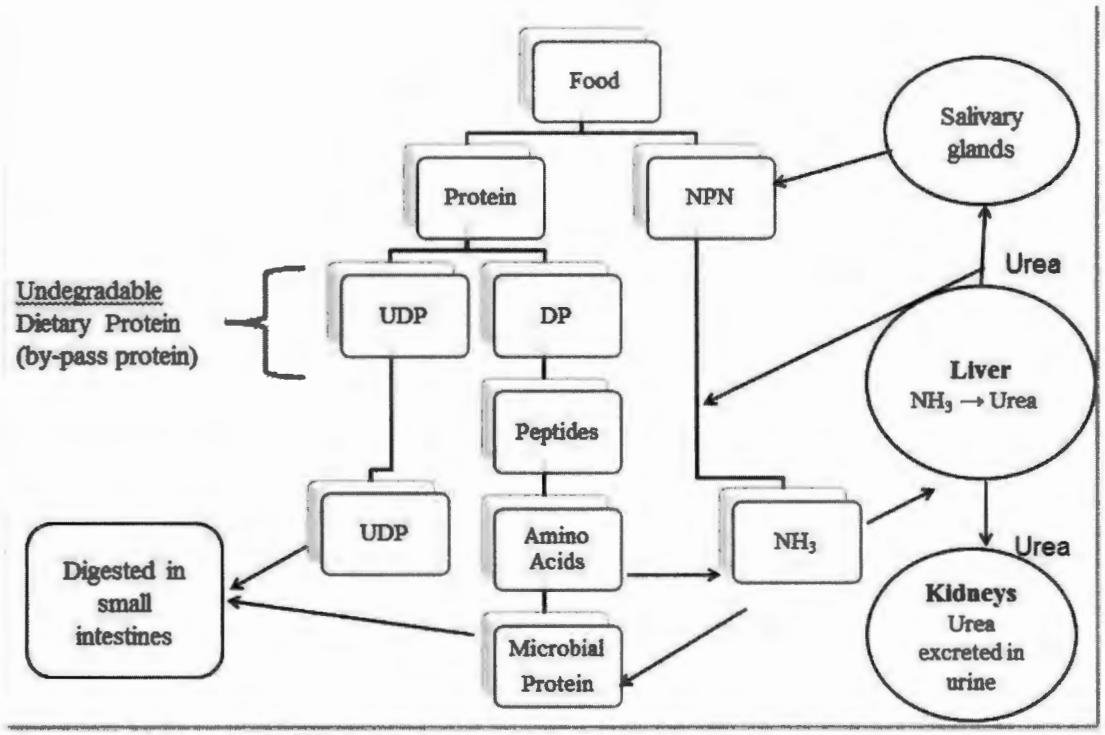


Figure 2.1. The fate of protein in the rumen and its immediate surroundings (Mlambo, pers. comm.)

Figure 2.1 shows how protein is degraded in the rumen of ruminant animals and also indicates how some of the proteins escape ruminal degradation. For example, when a protein diet is provided, proteolytic enzymes and microbes break it down into peptides or amino acids and then fermented to synthesize microbial protein which is absorbed in the digestive system. On the other hand, undegradable protein is excreted from the liver as urea through urination or can undergo nitrogen recycling.

2.7 Polyphenolics in ruminant nutrition and health

Classification of polyphenolics is based on their chemical structure and biochemical properties. They are categorised into tannin phenolics and non-tannin phenolics. The non-tannin phenolics have lower molecular weights and they are unlikely to form complexes and therefore cannot be bound by polyethylene glycol (PEG) (Mlambo *et al.*, 2009). The tannin phenolics have the ability to bind protein and carbohydrates and

consequently form complexes. Tannins are secondary plant compounds that either exhibit positive and negative nutritional effects when included in animal feeds. Tannins play a significant role in plants' defence mechanisms against environmental stresses or herbivory, and they act as toxins that eventually suppress voluntary intake (Aganga & Mosase, 2001). According to Barbehenn & Constabel (2011), tannins are formed to defend the plants against herbivores by producing toxic chemical compounds. According to Njidda (2010), a large number of plants produce secondary compounds which are not directly involved in the growing process of the plant but they serve as deterrents to insects and fungal attack and they can also affect animals and the nutritive value of the forages. Tannin phenolics are subdivided into two groups based on their structural type, known as, the hydrolysable and condensed tannins.

2.7.1 Hydrolysable tannins

Hydrolysable tannins (HT) are described as polyesters of phenolic acids (Makkar, 2003) and they are esterified to core molecules (Reed, 1995). They have a highly variable structure with different types of glycol and a range of cross-linkages between phenolic and gallic acids. When HTs are degraded in the rumen, the end-products are hepatotixin and nephrotoxin (Reed, 1995). Although hydrolysable tannins are degradable in the rumen, they are also likely to cause negative effects or toxicities to ruminants (Waghorn, 2008). Reed (1995) explained that HTs are widely distributed and in some *Acacia* species, they can go up to 200 g/kg in DM basis.

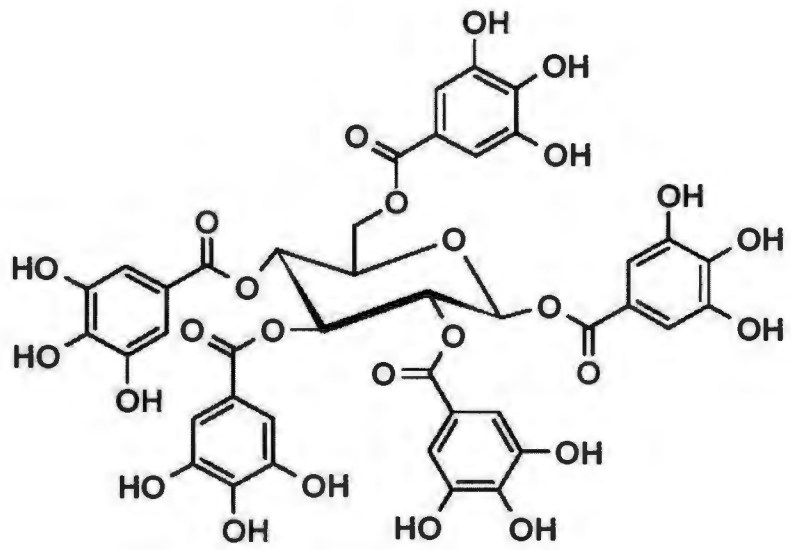


Figure 2.2. Structure of a hydrolysable tannin showing cross-linkages (Adapted from Barbehenn & Constabel, 2011)

Hydrolysable tannins become toxic when large amounts are consumed by ruminants with less opportunity for microbial population in the rumen to adapt (Waghorn, 2008). High consumption may reduce animal performance, and decrease the degradation of protein or carbohydrates and eventually causing death. Hydrolysable tannins can be easily broken down by enzymes or microorganisms and when gradually offered to ruminants, chances are that their use would be efficient.

2.7.2 Condensed tannins

Condensed tannins (CT), known as proanthocyanidins, are composed of oligomers and polymers bonded by carbon-carbon bonds and they can thrive well under anaerobic mammalian digestion (Waghorn, 2008). They are also classified as flavonoids based polymers (Makkar, 1995).

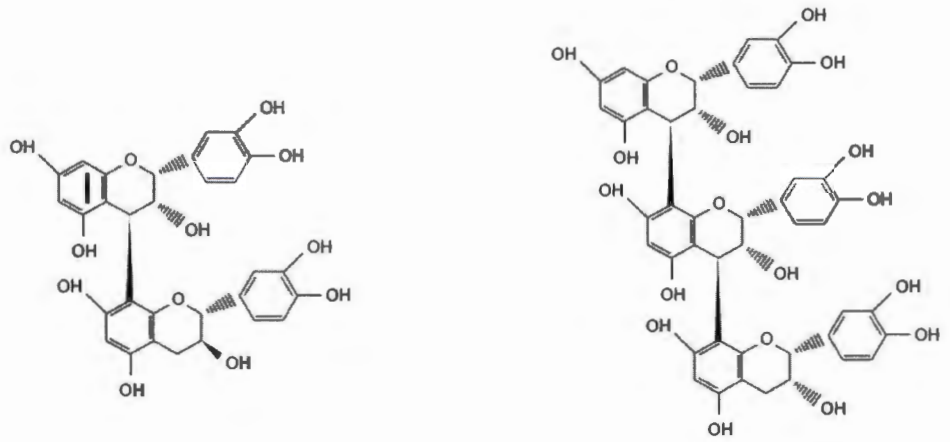


Figure 2.3. Structures of condensed tannins linked by carbon-carbon bonds (Adapted from Barbehenn & Constabel, 2011)

The role of CT in plant material is not known but their effects on ruminal digestion have been under investigation (Waghorn, 2008). When CTs bind to proteins or carbohydrates in the rumen, they become undegradable which then supports the ease flow of tannin-bound proteins into the lower tracts, where they can be disassociated and digested. According to Makkar *et al.* (1999), CTs vary in terms of their location, concentration and composition throughout the life cycle of plants. These tannin phenolics are diverse because of their intermolecular linkages, stereochemistry, monomers and polymers size. Condensed tannins accumulate in the epidermal and sub-epidermal layers of leaves and fruits in most tree species (Barbehenn & Constabel, 2011).

2.7.3 Beneficial effects of tannins

Tannins have the capacity to bind to proteins and increase the amount of by-pass protein through the rumen into the small intestine for degradation since in high concentration they reduce the utilisation of proteins in the rumen (Barbehenn & Constabel, 2011). The inability of the rumen to degrade protein bound with tannins

increases the amount of by-pass protein which is beneficial when ruminants are in high stages of production for example high-producing dairy cows. The ability of tannins to bind proteins in the rumen depends on their concentration and nutrient level (Barbehenn & Constabel, 2011). Tannins have the capacity to bind with proteins in an acidic to mildly environmental conditions resembled by that of the rumen, therefore, small intestines have a higher pH compared to that of the rumen and facilitates the disassociation of tannins from binding to proteins. Condensed tannins can prevent bloat and increase the flow of non-ammonia nitrogen and essential amino acids (Waghorn, 2008). Condensed tannins can act as bloat-guards especially when ruminants are exposed to lush and young pastures which would increase ruminal gas production above acceptable levels. Therefore, a provision of moderate tanniferous feeds can help prevent the incidence of bloat by reducing gas production, and produce substrates that are not available to microbes and also reduce *Streptococcus bovis* (Waghorn, 2008).

Condensed tannins reduce foaming in the rumen of protein-rich diets. These tannins have the ability to reduce methanogenesis, which is the production of methane, because they reduce microbial activity since they are toxic to most microbes or due to the defaunation of ruminal microbes therefore, the level of gases produced is reduced (Estell, 2010). Methane reduction is environmentally-friendly because methane gas is one of the green-house gases that have a negative effect onto the environment and largely contributing to global warming. According to Waghorn (2008), CTs are responsible for methane reduction per DM intake.

Condensed tannins improve the efficiency of nitrogen captured by rumen microbes and thus reducing urinary N losses (Mlambo *et al.*, 2008). For example, Waghorn (2008) stated that CTs would always maximise the flow of plant protein into the lower tracts and consequently reducing the absorption of ammonia in the rumen. The presence of

CTs in a diet can increase weight gain, wool growth and milk production (Barry & McNabb, 1999). Proanthocyanidins have a direct anthelmintic effect. Hoskin *et al.* (1999) reported that, for a given ruminant host animal, grazing on forages with high concentration of CTs had lowers the faecal egg count and worm burdens than those feeding on forages with low concentrations. The availability of proteins in the small intestine was determined by Mueller-Harvey & McAllan (1992) as responsible for increased immunological responses towards parasites, and sheep production had suffered economic losses as a result of the gastrointestinal nematodes. Feeding diets higher in CTs can be an affordable alternative to control gastrointestinal parasites (Athanasiadou *et al.*, 2001), since it reduces faecal egg counts and parasitic burdens (Akkari *et al.*, 2008). However, the anthelminthic effects of CTs vary due to the concentration and production of tannins, therefore, more investigation is required to understand the anthelmintic effect of tannins. Minho *et al.* (2008) stated that many studies are required to develop a commercial product based on browse-containing tannin extracts. These products can be used in large scale production systems as it has evidenced anthelmintic effects.

2.7.4 Negative effects of tannins

Although tannins have positive nutritional effects, they also possess negative effects, for example, they form complexes with protein and carbohydrates making them unavailable to the rumen microbes. The ability of tannins to form complexes contributes to their toxicological effects (Hagerman & Butler, 1981). When diets with high concentrations of tannins are provided to ruminants, they may result in increased incidences of metabolic disorders and toxicities (Reed *et al.*, 1990). Tannins can be toxic to ruminal microbes, which would consequently suppress their activities (Mlambo *et al.*, 2009). Scalhert (1991) has identified three mechanisms that cause tannin toxicity

to microorganisms, and they are as follows: enzyme inhibition and substrate deprivation, tannin action on membranes, and metal ion deprivation. Tannins may form indigestible complexes with cell wall carbohydrates by binding bacterial enzymes (Reed *et al.*, 1990). They also have corrosive effects to the small intestines because the disassociations of tannins from protein occur in the small intestine and large amount of tannins are disposed which causes corrosions. Condensed tannins can also affect the abomasal or intestinal mucosa and therefore, decreasing the absorption of the other nutrients (Reed, 1995). In support, Rittner & Reed (1992) stated that proanthocyanidins negatively affect the mucosa of the digestive tract, which decreases the absorption of nutrients. The toxicity of CTs to ruminants is difficult to separate from their profound effect onto the digestion of nutrients. Tannins decrease the absorption of essential amino acids particularly methionine and also growth rate (Butler *et al.*, 1986). Excessive consumption of feed containing high level of HTs may cause lesions with haemorrhagic gastroenteritis, liver necrosis, and kidney damage with proximal tubular necrosis (Filippich *et al.*, 1991). Acute intoxication may also result from an increased consumption of tannin-rich tree species that contain around 20% of HTs and could cause high mortality and morbidity in cattle and sheep. The exact toxic compound is still uncertain and unknown. Therefore, it is important to reduce the detrimental effects of tannins while maximising their beneficial effects for ruminant nutrition and this can be done by having more and concerted investigations directed to the concentration and biological activity of phenolics.

2.8 Ruminant feed evaluation techniques

2.8.1 Chemical analyses

Chemical analyses include many methods that are used to measure the primary and secondary chemical constituents of any plant material. It is critical to analyse the chemical composition and phenolics in order to know the amount of nutrients and tannins that the animals receive during consumption. Analysis of the chemical constituents of plant materials plays a pivotal role in terms of formulating rations and mixing different ingredients so as to come out with a balanced ration that contains all the nutrients required by the animals for maintenance, growth, reproduction and production.

Feed materials are chemically analysed according to AOAC (1999, method no. 976.06) for dry matter (DM), organic matter (OM), and total nitrogen (N); and also according to van Soest *et al.* (1991) for neutral detergent fibre (NDF), acid detergent fibre (ADF) using an ANKOM Fibre analyser. For many years, the Kjeldahl analysis has been a standard method in assessing the total nitrogen content of feedstuffs and the total N x 6.25 is an estimate of the soluble crude protein. Soluble protein is considered to be rapidly degraded to ammonia by the rumen microbial population. According to Licitra *et al.* (1996), it is considered as a true protein which is soluble in buffer at rumen pH, usually adjusted to 6.5. The van Soest technique is a modification of a proximate analysis and is acknowledged as the standard technique to analyse forages for ruminants. It uses improved methods for estimating fibre and protein values.

The guidelines from AgriLASA (1998) estimate the accurate mineral matter contained by feed materials. Soluble phenolics (SPh) are also chemically analysed using the Folin-Ciocalteau reagent and gallic acid as a standard and as described by Singleton &

Rossi (1965), while insoluble phenolics are chemically analysed according to Terrill *et al.* (1992). Tannin analyses are performed to determine the influence of tannins in protein digestion and nitrogen excretion in ruminants.

2.8.2 Buffer N solubility

A protein that has good qualities is the one that is degraded by rumen microbes and by-pass rumen degradation for further digestion in the abomasum (Garcia, 2013). Means of determining a good quality protein have been undertaken in several evaluation techniques to estimate the ruminal degradation of amino acids. *In vitro* and *in situ* bag techniques are methods that were largely used in the past in determining N degradation as it pertains to the loss of nitrogen. These two methods, however, have raised concerns on animal welfare and ethics (Mould *et al.* 2005). They are very complicated and costly as they require highly skilled technicians and animals that are surgically modified with a cannula. Buffer nitrogen solubility is one of the techniques that have been developed to determine the amount of nitrogen that would be soluble at rumen pH and eventually give the true protein fraction of the non-conventional protein feed (Licitra *et al.*, 1996). This concept evolved from the observation that a large percentage of soluble protein is degraded in the rumen and, as a consequence, a reduced amount of protein passes to the lower tract. According to NRC (2001), knowing the fraction of protein that is instantly degraded in the rumen is of major importance in ranking feedstuffs that have similar crude protein degradability. Buffer solubility is a technology that mimics the rumen environment as bicarbonate buffer is used to breakdown protein at a controlled temperature of 37°C and is measured using the modification of Licitra *et al.* (1996), the Daisy ^{II} Incubator (ANKOM Technology, Macedon, NY).

2.8.3 *In sacco* and *in vitro* rumen degradability techniques

In sacco (nylon bag) and *in vitro* methods are used to determine feedstuff degradability and the potential of rumen microbial population at different time intervals. The *in sacco* technique is described by Ørskov *et al.* (1980) as one of the methods used to analyse feeds in the early stages of determining and assessing their nutritional value. In this method, nylon bags are filled with sample feed and then incubated in the rumen of a fistulated ruminant at different time intervals and in order to produce measurable kinetic data on the degradation of feedstuffs. These bags are manufactured using polyester or nylon, having pores that are big enough to allow bacteria to enter the bag but small enough to keep the feed sample within the bags (30-50 µm). This method requires a trained person to perform and in order to have accurate results as data should be reproduced and replicated (Garcia, 2013). Tuah *et al.* (1996) reported limitations such as bag-pore size, sample weight washing methods and sample preparation because in this method, the samples are not exposed to chewing or regurgitation. The adherence of microorganisms at the early stages of the experiment can cause higher weights and affect results (McDonald, 1981). Although the nylon bag technique measures nutrient degradation accurately, a relatively new technology has been developed as an alternative to ensure fast, humane and less labour required. The ANKOM Daisy^{II} Incubator is the method used to simplify the *in sacco* method without having to use live animals. In most studies that were conducted, the ANKOM Daisy^{II} Incubator gave acceptable digestibility estimates of the feedstuff (Damiran *et al.*, 2002).

2.8.4 The *in vitro* ruminal gas production technique

The ANKOM^{RF} Gas Production System is a new technology designed to measure the kinetics of rumen microbial fermentation in an automated fashion by monitoring the gas

pressure within multiple modules and remotely recording the data in computer spread-sheets. This system is equipped with a temperature sensor and each module can also monitor the temperature of its environment. Fifty individual Modules can be included in the system to communicate information to a computer using radio frequency (RF) transmission. From the computer interface, the operator can control numerous variables such as data recording intervals and the automatic release of pressure through internal valves in each Module (ANKOM Technology, Operators' Manual). The method uses the Daisy^{II} Incubator to determine *in vitro* true degradability. Collected rumen inoculum passes through a cheese-cloth and all glassware is maintained at the temperature of 39°C and the feed samples are incubated in the rumen inoculum (ANKOM Technology Corporation, Fairport, NY). This method is designed as an alternative to the *in situ/ in sacco* method that uses fistulated animals which may raise concerns from the public.

2.8.5 Metabolism and feeding trials

Browse products are a valuable fodder source for ruminants during periods where grasses are dry. In a study conducted by Osuga *et al.* (2012), *Z. mucronata* leaves enhanced the nutritional value of low quality fibrous feeds and increased the total dry matter intake of grass hay with no goats showing health problems. Seeds of *Acacia nilotica* and fruits of *A. erioloba* may pass through the animals' digestive system in large proportions undigested. However, crushing or changing the physical state of these browse products before feeding can help in making the nutrients that are contained by seeds and fruits available to the animals (Aganga *et al.*, 1998). According to Estell (2010), the metabolism by which plant secondary metabolites affect digestion and metabolism differ among compounds, for example when feeding foliage with HTs, they are efficiently absorbed but may exhibit toxic effects at tissue levels. However, when feeding foliage containing CTs, they may be poorly absorbed and exert toxic effects.

Mlambo *et al.* (2009) reported that the tannin content in the tree species interferes with the digestion and metabolism of feed, which disturbs and reduces protein degradability and also limits *in vitro* fermentation.

2.8.6 The near infrared reflectance spectroscopy

Near infrared reflectance spectroscopy (NIRS) has the capacity to provide accurate estimates of feed components in a cheap and most rapid way. According to Azzouz *et al.* (2003), NIRS accurately analyses the qualitative and quantitative aspects of the chemical components of any feed material. The predictions resulting from the use of NIRS allows farmers to formulate a balanced ration that would meet the desired levels of production, although it is unlikely that farmers would adopt NIRS as a feed evaluation technique due to its technical challenges and cost. Most farmers rely on ripe stage as an indicator to determine readiness of the leaves for collection, feeding and storage for future usage. The ripe stage is usually based on colour of leaves. When leaves change colour, they are considered to be matured and ready for usage (Kusumiyati *et al.*, 2008) but their chemical composition is not known. Therefore, by the use of NIRS, the chemical composition of the leaves can easily be estimated and quantified accurately and rapidly. According to Kusumiyati *et al.* (2008), information from NIRS on diets' quality parameters can be integrated in calibration and prediction models. If the quality parameters can establish a strong correlation, then models can be used to determine the optimum time of harvesting, than taking the NIRS machinery to the fields which might be rather expensive and risky. The use of NIRS can minimise the rejection of leaf-meals from sorting machine and can allow farmers to efficiently utilise resources and labour (Azzouz *et al.*, 2003).

According to Wheeler *et al.* (1996), NIRS has been a reliable technique for estimating the feed quality. Methods of analysing feed to estimate their qualities have been widely used, which includes two approaches such as the direct analysis of feed before feeding by the animals, and the indirect analysis of the faecal spectra after feeding. Azzouz *et al.* (2003) suggested that NIRS is exceptional and requires no reagent to perform. It generates results in a short time, it is cheap and easy to perform though the equipment is expensive and requires no sample processing hence it is efficient. Kusumiyati *et al.* (2008) stated that after-harvesting, NIRS can be used as a fast and non-destructive technique for sorting out or grading leaf-meals, based on desired quality attributes. By combining exceptional accuracy across a broad wavelength range of 400 to 2500 nm with full compatibility of all existing and future FOSS solutions, this instrument may lay a foundation for new levels of excellence in analysis. Tree breeding programs require large numbers of trees to be screened in the most rapid and cost effective way. Indeed, NIRS analysis has certainly assisted in making it possible to predict a large number of traits.

The NIRS analysis has addressed the issue of cost because it can provide reliable estimates of feed chemical composition at about one fifth of the cost of conventional chemical analyses to determine the amount of structural fibre, soluble carbohydrate, crude protein, lipid and ash. Near Infrared Reflectance Spectroscopy can also predict the digestible and metabolisable energy value of a feed (Ulyatt *et al.* 1995). The NIRS analysis is increasingly used to balance rations for dairy cattle. Conventional chemical analyses of feed composition are time-consuming whereas with NIRS, an analysis can be completed within 2 - 3 minutes on suitably prepared samples. Forages can also be analysed fresh, dried, or dried and ground, however every sample preparation produces

a different spectrum (Ulyatt *et al.* 1995), so the type of preparation should be defined before calibration of the instrument database.

2.9 Summary of the literature review

Literature have fully emphasised that browse leaves have the potential to be used as a source of protein to supplement poor quality forages. The utilisation of these browse leaves is however influenced by secondary compounds such as polyphenolics. Results from different scholars have shown variations in the nutritional value of browse leaves which are due to growth environments. No research have been done in the rangelands of Molelwane and Masuthle, therefore, this continues the search for alternative cheap sources of proteins that can be used during the dry season when grasses are limiting in protein and have high lignin content which negatively affect feed digestibility.

2.10 References

Aganga, A.A. & Mosase, K.W., 2001. Tannin content, nutritive value and dry matter digestibility of *Lonchocarpus capassa*, *Zizyphus mucronata*, *Sclerecarya birrea*, *Kirkia acuminata* and *Rhus lancea* seeds. Anim. Feed Sci. Technol. 91: 107 – 113.

Aganga, A.A., Tsopito, C.M. & Adogla-Bessa, T., 1998. Feed potential of *Acacia* species to ruminants in Botswana. Arch. Zootec. 47, 659-668.

Aganga, A.A., Tsopito, C.M., Yeboah, S.O., Mokgoko, C. & Manne, S.N., 2003. Evaluation of the chemical composition of some locally available *Acacia* seeds as animal feed in Botswana. World Anim. Rev. 23, 135-136.

Agri Laboratory Association of Southern Africa (AgriLASA), 1998. Feed and Plant Analysis Methods. AgriLASA, Pretoria, South Africa.

Akkari, H., Salem, H.B., Gharbi, M., Abidi, S. Darghouth, M.A., 2008. Feeding *Acacia cyanophylla* Lindl. Foliage to Barbarine lambs with or without PEG: Effect on the excretion of gastro-intestinal nematode eggs. Anim. Feed Sci. Technol. 147, 182 -192.

Amigot, S.L., Fulgueira, C.L., Bottai, H., Basilico, J.C. & Basilico, M.L.Z, 2005. Estategias para la calidid de forrajes. In: Proceedings of Xth Argentine Congress of Micology, Buenos Aires, Argentina. Pp 125.

AOAC, 1999. Method number 976.06. In Official Methods of Analysis of AOAC International, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. Vet. Parasitol. 99, 205–219

Azzouz, T., Puigdoménech, A., Aragay, M. Tauler, R., 2003. Comparison between different pre-treatment methods in the analysis of forage samples using near-infrared diffuse reflectance spectroscopy and partial least-squares multivariate calibration method. Chimica Acta. 484, 121 – 134.

Barbehenn, R.V. & Constabel, C.P., 2011. Tannins in Plant-Herbivore Interactions. Phytochemistry. 72, 1551 – 1565.

Barry, T.N. & McNabb, W.C., 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Br. J. Nutr. 81, 263 - 272.

Boudet, A.M., 1998. A new view of lignification. Trends Plant Sci. 3, 67 - 71.

Brenan, J. P. M., 1983. Manual on taxonomy of *Acacia* species: present taxonomy of four species of *Acacia* (*A. albida*, *A. senegal*, *A. nilotica*, *A. tortilis*). FAO. Rome, Italy.

Butler, L.G., Rogler, J.C., Mehansho, H. & Carlson, D.M., 1986. Dietary effects of tannins. Plant Flavanoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships. Alan R. Liss, *NY*. Pp. 141.

Coe, M., 1998. Some aspects of the interaction between Mammalian herbivores and *Acacia erioloba*. T. Roy. Soc. S. Afr. 53, 141-147.

Cook, B.G., Pengelly, B.C., Brown, S.D., Donnelly, J.L., Eagles, D.A., Franco, M.A., Hanson, J., Mullen, B.F., Partridge, I.J., Peters, M. & Schultze-Kraft, R., 2005. Tropical Forages. CSIRO, DPI & F(Qld), CIAT & ILRI, Brisbane, Australia.

CSIRO, 2007. Nutrient requirements of domesticated ruminants. CSIRO Publishing. Collingwood, Australia. Pp 1-270.

Cudjoe, N. & Mlambo, V., 2014. Buffer nitrogen solubility, *in vitro* ruminal partitioning of nitrogen and *in vitro* ruminal biological activity of tannins in leaves of four fodder tree species. J. Anim. Physiol. Anim. Nutr. 98, 722 - 730.

Damiran, D., Delcurto, T., Bohnert, D.W., Pulsipher, G.D. & Findholt, S.L., 2002. "Comparison of Techniques and Grinding Size to Estimate Digestibility of Forage Base Ruminant Diets." AM. Soc. Anim. Sci. 53, 341 – 344.

Davidson, L., 1981. *Acacias*, a field guide to the identification of the species of southern Africa. Centaur Publishers, Johannesburg, South Africa.

Ecocrop, 2012. Ecocrop database. Food & Agricultural Organisation. Website accessed on 12 March 2014: http://ecocrop.fao.org/ecocrop/srv/en/home.

Ecocrop, 2014. Ecocrop database. FAO, Rome, Italy.

Ellis, R.P., 2003. *Ziziphus mucronata* Willd.. In: Ecoport database. Ecoport.

Estell, R.E., 2010. Coping with shrub secondary metabolites by ruminants. Small Rumin Res. 94, 1 – 9.

Fagg, C.W. & Mugedo, J.Z.A., 2005. *Acacia nilotica* (L.) Wild. Ex Delile. In: Jansen, P.C.M. & Cardon, D. (Eds). Record from Protabase. Plant Resources of Tropical Africa (PROTA), Wageningen, Netherlands.

Filippich, L.J., Zhu, J. & Alsalmi, M.T., 1991. Hepatotoxic and nephrotoxic principles in Terminalia oblongata. Res. Vet. Sci. 50: 170.

Garcia, S.N., 2013. Chemical composition, nitrogen buffer solubility and simulated rumen degradability of potential non-conventional protein supplements. A thesis. Pp.: 1 – 95.

Hagerman, A.E. & Butler, L.G., 1981. The specificity of proanthocyanidin-protein interactions. J. Biol. Chem. 256, 4494.

Hassen, A., Rethman, N.F.G. & van Niekerk, W.A., 2009. A note on the potential nutritive value of *Ziziphus mucronata* (buffalo thorn) foliage during different seasons. Afr. J. Range & Forage Sci., 26(2), 103 – 105.

Heuzé, V. & Tran, G., 2014. Buffalo thorn (*Ziziphus mucronata*). Feedipedia.org. A programme by INRA, CIRAD, AFZ & FAO.

Hoskin, S.O., Barry, T.N., Wilson, P.R., Charleston, W.A.G. & Hodgson, J., 1999. Effects of reducing anthelmintic input upon growth and faecal egg and larval counts in young farmed deer grazing chicory (*Chicorium intybus*) and perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens)* pasture. J. Agr. Sci. 132, 335 - 345.

Kibria, S.S., Nahar, T.N., Mia, M.M., 1994. Tree leaves as alternative feed resource for black Bengal goats under stall-fed conditions. Small Rumin Res. 13, 217 – 222.

Kusumiyati, A., Akinaga, T. & Tanaka, M., 2008. Multivariate approach to the measurement of tomato maturity and gustatory attributes and their rapid assessment by Vis-NIRS spectroscopy. J. Food Agr. Environ. 6, 327 – 332.

Leistner, O.A., 1961. On the dispersal of *Acacia giraffae* by game. Koedoe. 4, 101 - 104.

Leng, R.A., 1990. Factors affecting the utilisation of 'poor-quality' forages by ruminants particularly under tropical conditions. Nutr. Res. Rev. 3, 277 – 303.

Licitra, G., Hernandez, T.M. & Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347 - 358.

Maier, W., Khoza, T., Harmse, N., Wingfield, B.D. & Wingfield, M.J., 2006. A disease epidemic on *Ziziphus mucronata* in the Kruger National Park caused by *Coniodictyum chevalieri*. Stud. Mycol. 55, 279 - 288.

Makkar, H.P.S., 1995. Quantification of tannins: A Laboratory Manual, 2nd Edition. International Centre for Agricultural Research in the Dry Areas. Aleppo, Syria.

Makkar, H.P.S., Blummel, M. & Becker, K., 1999. Potential and limitations of *in vitro* gas methods for studying the effects of plant defensive compounds on rumen fermentation. In: Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed. Eds Jansman, A.J.M., Huisman, J. & Van der Poel, A.F.B., Wageningen Press, Wageningen. Pp. 173 – 178.

Makkar, H.PS., 2003. Quantification of Tannins in Tree and Shrub Foliage. A Laboratory Manual. Pp. 1 - 99.

Mazibuko, N., 2007 *Ziziphus mucronata* Willd.. South African National Biodiversity Institute (SANBI), South Africa.

McDonald, I. 1981. A revised model for the estimation of protein degradability in the rumen. J. Agr. Sci., 96, 251 - 252.

Minho, A.P., Bueno, I.C.S., Louvandini, H., Jackson, F., Gennari, S.M. & Abdalla, A.L., 2008. Effect of *Acacia molissima* tannin extract on the control of gastrointestinal parasites in sheep. Anim. Feed Sci. Technol.147, 172 - 181.

Mlambo, V., Mould, F. L., Smith, T., Owen, E., Sikosana, J. L. N. & Mueller-Harvey, I., 2009: *In vitro* biological activity of tannins from *Acacia* and other tree fruits: correlations with colorimetric and gravimetric phenolic assays. S. Afr. J. Anim. Sci. 39, 131 - 143.

Mlambo, V., Mould, F.L., Sikosana, J. L. N., Smith, T. Owen, E. & Mueller-Harvey, I., 2008. Chemical composition and *in vitro* fermentation of tannin-rich tree fruits. Anim. Feed Sci. Technol. 140, 402 - 417.

Mould, F.L., Kliem, K.E., Morgan, R., & Mauricio, R.M., 2005. "*In Vitro* Microbial Inoculums: A Review of its Function and Properties." Anim. Feed Sci. Technol. 123, 31 – 50.

Mueller-Harvey, I. & McAllan, A.B., 1992. Tannins: their biochemistry and nutritional properties. Adv. Plant Cell Biochem. Biotechnol. 1, 151 - 217.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle, 7th ed. Washington D.C.: National Academy Press.

Ngwa, A.T., Nsahlai, I.V. & Bonsi, M.L.K., 2000. The potential of legume pods as supplements to low quality roughage. J. Sci. Food Agric. 30, 107 - 108.

Njidda, A.A. & Nasiru, A., 2010. *In vitro* gas production and dry matter digestibility of tannin-containing forages of semi-arid region of North-Eastern Nigeria. Pakistan J. Nutr., 9, 60 – 66.

Njidda, A.A. & Olatunji, E.A., 2012. Chemical composition, anti-nutritive substances and in situ digestion kinetics of four Ziziphus species leaves used as fodder for ruminants in semi-arid zone of Nigeria. J. Nat. Sci. Res. 2(7), 79 – 87.

Njidda, A.A., 2010. Chemical Composition, Fibre Fraction and Anti-Nutritional Substances of Semi-arid Browse Forages of North-Eastern Nigeria. Nigerian J. Basic Appl. Sci. 18(2), 181 - 188.

Nsahlai, F.N., Fon, F.N. & Basha, N.A.D., 2011. The effect of tannin with and without polyethylene glycol on *in vitro* gas production and microbial enzyme activity. S. Afri. J. Anim. Sci. 41:4.

Ondiek, J.O., Abdulrazak, S.A. & Njoka, E.N., 2010. Performance of growing Small East African Goats offered Rhodes grass hay and supplemented with a 1:1 mixture of *Maerua angolensis*: *Zizyphus mucronata* leaf browses. Livest. Res. Rural Dev. 22(9), 174.

Ørskov, E.R., Deb Hovell, F.D.& Mould, F., 1980. "The Use of the Nylon Bag Technique for the Evaluation of Feedstuffs." Trop. Anim. Prod. 5, 195 – 213.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Anthony, S., 2009. Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre. Kenya.

Osuga, I.M., Abdulrazak, S.A., Muleke, C.I. & Fujihara, T., 2012. Effect of supplementing Rhodes grass hay (*Chloris gayana*) with *Berchemia discolour* or *Zizyphus mucronata* on the performance of growing goats in Kenya. J. Anim. Physiol. Anim. Nutr. 96, 634 – 639.

Osuga, I.M., Maindi, C.N., Nishino, N., Abdulrazak, S.A., Ichinohe, T. & Fujihara, T., 2007. Potential nutritive value and tannin bioassay of selected *Acacia* species from Kenya. Anim. Sci. J. 79, 528 – 589.

Reed, J. D., Soller, H. & Woodward, A., 1990. Fodder tree and straw diets for sheep: Intake, growth, digestibility and the effects of phenolics on nitrogen utilisation. Anim. Feed Sci. Technol. 30, 39.

Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 73, 1516 - 1528.

Rittner, U. & Reed, J.D., 1992. Phenolics and *in vitro* degradability of protein and fibre in West African browse. J. Sci. Food Agric. 58, 21.

Roodt, V., 1998. Trees and shrubs of the Okavango Delta, Medicinal uses and nutritional value. Gaberone, Botswana. The shell field guide series: Part 1. Pp. 815.

Rothaugue, A., Kaendji, G. & Nghikembua, M.L., 2003. Forage preference of Boer goats in the highland savannah during the rainy season II: nutritive value of the diet. Agricola. 13, 43 – 48.

Russell, J.B., Sharp, W.M. & Baldwin, R.L., 1979. The effect of pH on maximum bacterial growth rate and its possible role as a determinant of bacterial competition in the rumen. J. Anim. Sci. 48, 251 – 255.

Scalhert, A. 1991. Antimicrobial properties of tannins. Phytochemistry. 30, 3875.

Seymour, C. & Suzanne, M., 2003. Collation and overview of research information on *Acacia erioloba* (camel thorn) and identification of relevant research gaps to inform protection of the species. Res. Gaps. 89, 1 - 32.

Singleton, V.L. & Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144 - 158.

Smith, N., 1999. Guide to the *Acacias* of South Africa. Briza Publications, Pretoria, South Africa.

Tanner, J.C., Reed, J.D. & Owen, E., 1990. The nutritive value of fruits (pods with seeds) from four *Acacia* spp. compared with extracted *Noug* (*Guizotia abyssinica*) meal as supplements to maize stover for Ethiopian highland sheep. Anim. Prod. 51, 127 - 133.

Tefera, S., Mlambo, V., Dlamini, B.J., Dlamini, A.M., Koralagama, K.D.N. & Mould, F.L., 2008. Chemical composition and *in vitro* ruminal fermentation of common tree forages in the semi-arid rangelands of Swaziland. Anim. Feed Sci. Technol. 142, 99 - 110.

Terblance, M., Pienaar, J.G., Bigalke, R. & Vahrmeyer, J., 1967. *Acacia nilotica* (L.) del. Susp. Kraussiana (Benth.) Brenan. as a poisonous plant in South Africa. J. S. Afr. Vet. Med. Assoc. 38, 57 - 63.

Terrill, T.H., Rowan, A.M., Douglas, G.B. & Barry, T.N., 1992. Determination of extractable and bound condensed tannin concentration in forage plants, protein concentrate meals and cereal grains. J. Sci. Food Agr. 58, 321 - 329.

Timberlake, J., Fagg, C. & Barnes, R., 1999. Field Guide to the *Acacia* of Zimbabwe. CBC Publishing, Harare, Zimbabwe.

Tuah, A.K., Okai, D.B., Øskorv, E.R., Kyle, D., Wshand, J.F., Greenhalgh, D. Obese, F.Y. & Karikari, P.K., 1996. "*In sacco* Dry Matter Degradability and *In vitro* Gas Production Characteristics of Some Ghanaian Feeds." Livestock Research for Rural Development 8 (1). Accesses January 13, 2015. http://www.lrrd.org/lrrd8/1/tuah.htm/

Ulyatt, M.J., Lee, J. & Corson, D., 1995. Assessing feed quality. Ruakura Farmers Conference.47, 59 – 62.

Van Hoven, W. & Furstenburg, D., 1992. The use of purified condensed tannin as a reference in determining its influence on rumen fermentation. Comparative Biochemistry & Physiology Part A: Physiology. 101, 381 – 385.

Van Soest, P.J., 1987. Nutritional ecology of the ruminant. Cornell University Press. Ithaca, New York, USA.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583 - 3597.

Van Wyk, A.E. & van Wyk, P., 1997. Field guide to trees of southern Africa. Struik, Cape Town.

Van Wyk, B., van Wyk, P. & van Wyk, B.E., 2000. Photographic guide to trees of southern Africa. Briza Publications, Pretoria, South Africa.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production- Progress and challenges. Anim. Feed Sci. Technol. 147, 116 – 139.

Wheeler, R.A., Chaney, W.R., Johnson, K.D. & Buttler, L.G., 1996. *Leucaena* forage analysis using near infrared reflectance spectroscopy. Anim. Feed. Sci. Technol. 64, 1-9.

3 CHEMICAL COMPOSITION OF LEAVES FROM *ACACIA ERIOLOBA*, *A. NILOTICA* AND *ZIZIPHUS MUCRONATA* TREES HARVESTED FROM TWO SITES

Abstract

The study was designed to investigate the impact of different growth environments on the chemical composition of leaves from *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* tree species. Leaves from the mentioned tree species were harvested from Molelwane private farm and Masuthle communal grazing land and then analysed for dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), total nitrogen (N), minerals, soluble phenolics and condensed tannins. Leaves growing at the two sites were shown to have equal potentials to be used as sources of protein supplements because site did not significantly (P >0.05) affect crude protein (CP) content of the browse leaves. The highest CP content was found in leaves of *Z. mucronata* (177.7 g/kg DM), followed by *A. erioloba* (150.6 g/kg DM) and *A. nilotica* (127.6 g/kg DM). The soluble phenolics (SPh) of tree leaves showed significant (P <0.05) differences between the two sites. Leaves harvested from Masuthle had a higher SPh (44.58 g TAE/kg DM) content compared to those harvested from Molelwane (29.803 g TAE/kg DM). Leaves from Molelwane had a higher Ca content (6.51 g/100 g DM) compared to Masuthle (4.47 g/100 g DM). *Acacia nilotica* leaves had the lowest potential to be used for supplementary strategies because they had the lowest CP and the highest CT contents compared to the other two tree species. On the other hand, leaves of *Z. mucronata* had the greatest potential to be used as a protein supplement for ruminant animals because of their highest CP content. In both sites, *A. nilotica* leaves had lower NDF content compared to *A. erioloba* and *Z. mucronata* leaves, which did not differ. It was therefore, concluded that leaves harvested from

Molelwane had a higher nutritional value potential than the ones from Masuthle because they had higher N and mineral contents but lower in phenolics.

3.1 Introduction

Chemical composition alone cannot be used to accurately predict the feed value of forage but is an indicator of the level of nutrients that can potentially be available to ruminant animals (Norton & Poppi, 1995). The nutritive value of any feed substrate depends on the balance between the nutritive components of the feed source, the digestibility of its nutrients, the metabolism of absorbed nutrients and the amount of nutrients consumed (Aganga *et al.*, 2003). According to Norton & Poppi (1995), plants develop more fibre as they grow because they deposit more cell walls. Norton (1994) stated that leaves with NDFs lower than 35% are highly digestible, which therefore brings the need to quantify the nutritional contents of feed as a way to recommend optimum feeding to livestock. Cudjoe & Mlambo (2014) elaborated that the chemical composition of leaves vary due to stage of growth of plants, and the digestibility of browse products may be moderate enough to supply adequate amounts of nutrients to animals. Ruminants are naturally capable of digesting fibrous diets, but they are limited to an extent in utilising lignin. It is then important to quantify the lignin content before any feed is given to animals.

Browse leaves also develop lignin during growth (Norton & Poppi, 1995) but the actual amount needs investigation for proper utilisation. Leaves of *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* tree species are thought to be nutrient supplements for ruminants feeding on poor quality forages. These browse trees remains green and nutritious during the dry season when grasses are dry and low in nutrient content. During the dry season, protein is a major limiting nutrient that these browse leaves can

provide. Tefera *et al.* (2008) showed that the crude protein (CP) content differed considerably among *Acacia* species and soil type had no apparent effect on levels of any chemical component. The nutritive value of browse products is largely unknown due to variations with growth environments and as well as tree species. Lukhule & van Ryssen (2000), reported that the chemical composition of browse leaves have slight variations within stages of growth or season. This study, therefore, shed light on the variation of the nutritive value of important browse products as evaluated through chemical analysis. Therefore, the broad objective of the current study was to investigate the chemical composition of leaves from *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from two different growth environments. The alternative hypothesis was that tree species and growth environment cause variation in nutritional parameters as assessed by chemical analysis.

3.2 Material and Methods

3.2.1 Description of the harvesting site

Harvesting of samples was conducted in two growth environments; the North-West University Research Farm at Molelwane and the Masuthle village communal grazing land, both sites being located in the semi-arid region of the North-West province of South Africa but with a distance of 40 km between each other. These two sites are found at an altitude of 1500 meters above sea level. The geographical coordinates of Molelwane are 25°85'00" S, 25°63'33 "E, while Masuthle is located 25° 53' S, 31° 49' E". In both areas, temperatures range from a minimum of 3°C to a maximum of 39°C, and can sometimes drop to -3°C especially during the winter nights. The annual rainfall around the two areas is approximately 300 - 600 mm, although most of the rain is received between November and February. The vegetation type in both areas resembles

that of a Savannah biome. Molelwane is a private farm with goats and cattle that are mostly reared on commercial feed. These animals do not frequently utilise the natural pasture and browse in the farm. Masuthle is an open village where different kinds of herbivores all have unlimited access to the communal rangeland. In this village, there is no controlled grazing and soil erosion is high since it is an open land where people and animals move around at any given time. The grazing pressure in Masuthle is much greater than in Molelwane.

3.2.2 Harvesting of the leaves

Fresh leaves from 10 randomly selected individual trees, each of *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* tree species, were harvested from the two sites by hand. Individual trees were selected from within a marked 100 × 100 m area in each site. Each of the 60 samples was stored separately in labelled brown paper bag. Labelling was done according to the harvesting site, tree species, and tree number. Samples were immediately taken to the Animal Research laboratory at the North-West University Farm, where they were dried to constant weight in an oven set at a temperature of 60°C. The oven-dried leaves were then ground (Polymix PX-MFC 90 D) to pass through a 2 mm sieve for all chemical components except for phenolics, whose determination was carried out on samples milled to pass through a 1 mm sieve. Milled samples were kept in their respective labelled sample bottles pending nutritional assessment.

3.2.3 Chemical analyses

Milled leaf samples from *A. erioloba*, *A. nilotica* and *Z. mucronata* were analysed for dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), total nitrogen (N), minerals (calcium (Ca), phosphorus (P), sodium (Na),

sulphur (S), silicon (Si), magnesium (Mg), iron (Fe), cobalt (cobalt), copper (Cu), manganese (Mn), zinc (Zn)) and molybdenum (Mo)), soluble phenolics and condensed tannins in the Animal Science laboratory at Molelwane and the Animal Health laboratory at the Centre for Animal Health Studies.

3.2.3.1 Proximate components

For laboratory dry matter (DM) determination, approximately 1 g of each leaf sample was placed into pre-weighed crucibles and placed in an oven set at 105°C for 12 hours. The loss in weight was measured as moisture content and DM was calculated as the difference between the initial sample and moisture weights. Organic matter content (OM) was determined by ashing the dried samples in a muffle furnace set at 600°C for 6 hours. The loss in weight was measured as organic matter (OM) content and the residue as ash. Total nitrogen content was determined by the standard macro-Kjeldahl method (AOAC 1999, method no. 984.13) and was converted to crude protein by multiplying the percentage N content by a factor of 6.25 and expressed in g/kg DM. Neutral detergent fibre (NDF) and in full first ADF were determined by refluxing 0.45 g of samples with neutral detergent and acid detergent solutions respectively, for 1 hour and using the ANKOM^{2000} Fibre analyser (ANKOM Technology, New York) and according to van Soest *et al.* (1991). A heat-stable bacterial α-amylase but not sodium sulphite was used for the NDF analysis. The fibre fractions were then expressed in g/kg DM inclusive of the residual ash.

3.2.3.2 Mineral analyses

Mineral content was analysed in the Animal Health laboratory using the dry ashing macro and trace minerals methods, following the guidelines provided by the Agri-Laboratory Association of Southern Africa (AgriLASA, 1998). Samples that were used

to determine the DM were further incinerated in a muffle furnace for 12 h. The ash was weighed and digested with 1 mL of 55% nitric acid and 10 mL of 32% hydrochloric acid using a Microwave Reaction System Model 3000. Samples were digested for 45 minutes, cooled, and transferred into respective volumetric flasks (100 mL), which were eventually topped-up with distilled water and left standing for 24 h to allow the sediment to settle down. After 24 h, samples were slowly transferred to McCartney bottles without disturbing the sediment. The concentrations of Ca, P, Na, S, Si, Mg, Mn, Fe, Co, Cu, Zn and Mo were then determined using an ICP Mass Spectrometer (Perkin-Elmer, 1982, NexION 300Q). Macrominerals were presented as g/100 g DM while microminerals were presented as mg/kg DM.

3.2.3.3 Soluble phenolics

Soluble phenolics (SPh) were estimated using the Folin-Ciocalteau method (Makkar, 2003) after extracting a 200 mg sample three times (5 min at a time) with a 10 mL aqueous acetone (7:3 v/v, acetone:water). After extraction, a 0.25 mL Folin and Ciocalteau reagent (2N) was mixed with 0.02 mL of the acetone extract in a test-tube and then 1.25 mL of sodium carbonate (20%) was added to the mixture and vortexed. The mixture was allowed to react for 40 minutes, after which absorbance was measured at 725 nm wavelength using a spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments) (Makkar, 2003). Tannic acid (Makkar, 2003) was used to develop a standard curve from which the concentration of soluble phenolics in leaf samples was predicted. Soluble phenolics results were therefore, expressed as tannic acid equivalents (TAE).

3.2.3.4 Condensed tannins

The same aqueous acetone (7:3 v/v, acetone:water) leaf extract (0.5 mL) was used to assay for soluble condensed tannins (SCT) using the modified butanol-HCl reagent

(95:5 v/v) (Porter *et al.*, 1986). The extract described above was pipetted into a test-tube and 3 mL of butanol-HCL reagent was added. The test-tube was then closed and placed on a heating block set at 100°C for 1 h. After 1 h, the test-tubes were cooled to room temperature and absorbance was measured at 550 nm wavelength using a spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments). Soluble condensed tannin concentration was reported as absorbance units (AU) per 200 mg sample (Makkar, 2003).

3.2.4 Statistical analysis

Chemical composition of *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species was analysed using the general linear models (GLM) procedure of SAS version 9.3 (SAS, 2010). Data were analysed based on a two-way factorial treatment design within a completely randomised experimental design. Main effects were tree species (*A. erioloba*, *A. nilotica* and *Z. mucronata*) and growth environment (Molelwane and Masuthle sites). The linear statistical model was as follows:

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

Where, Y_{ijk} = dependant variable, μ = population mean, S_i = effect of tree species, GE_j = effect of growth environment, $(S \times GE)_{ij}$ = effect of interaction between tree species and growth environment, and E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P < 0.05$. Least squares means were compared using the probability of difference (pdiff) option in the lsmeans statement of SAS.

3.3 Results

3.3.1 Proximate composition

The chemical components (DM, ASH, OM, NDF, ADF and CP) of leaves harvested from *Acacia erioloba*, *A. nilotica* and *Z. mucronata*, expressed on a DM basis, are shown in Table 3.1. Site had no significant effect on all chemical components, while a significant effect of tree species was observed for all the chemical components. There was an interaction (P <0.05) between site and tree species in terms of NDF and ADF content of leaves. The laboratory DM content of leaves harvested from the three tree species ranged from 929.5 to 950.1 g/kg.

Table 3.1. Chemical components of leaves from *Acacia erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from two different sites (Molelwane and Masuthle)

	Tree species			
[1]Components	[2]AE	[3]AN	[4]ZM	SEM
DM (g/kg)	950.1[b]	929.5[a]	931.1[a]	1.915
ASH (g/kg DM)	55.9[a]	48.5[a]	79.1[b]	5.200
OM (g/kg DM)	894.2[b]	881.1[b]	851.9[a]	5.438
CP (g/kg DM)	150.6[b]	127.6[a]	177.7[c]	5.603

[1]Parameters: DM = dry matter; OM = organic matter; CP = crude protein

[2]AE = *Acacia erioloba*; [3]AN = *Acacia nilotica*; [4]ZM = *Ziziphus mucronata*

[a,b,c] For all components except NDF, in a row, different superscripts denote significant differences (P <0.05) between tree species

Table 3.1 shows that leaves from *Acacia erioloba* had a higher DM content than those of *Acacia nilotica* and *Ziziphus mucronata*, which did not differ (P >0.05). In both sites, there was no significant (P >0.05) difference between the leaves of *A. nilotica* and *Z. mucronata*. Leaves of *Z. mucronata* had the highest ash content as compared to those of *A. erioloba* and *A. nilotica*, which did not differ (P >0.05).

Figure 3.1 (a) shows that, in both sites, leaves from *A. erioloba* had the same NDF content as those of *Z. mucronata*. Both tree species had higher NDF contents compared to *A. nilotica*. In Molelwane, leaves of *A. erioloba* had the highest ADF content (413.0 g/kg DM) followed by *A. nilotica* (379.9 g/kg DM) and the least was *Z. mucronata* (189.7 g/kg DM). In Masuthle, the ADF content was the same (P >0.05) for *A. erioloba* and *A. nilotica*, but was higher (P <0.05) than that of *Z. mucronata*.

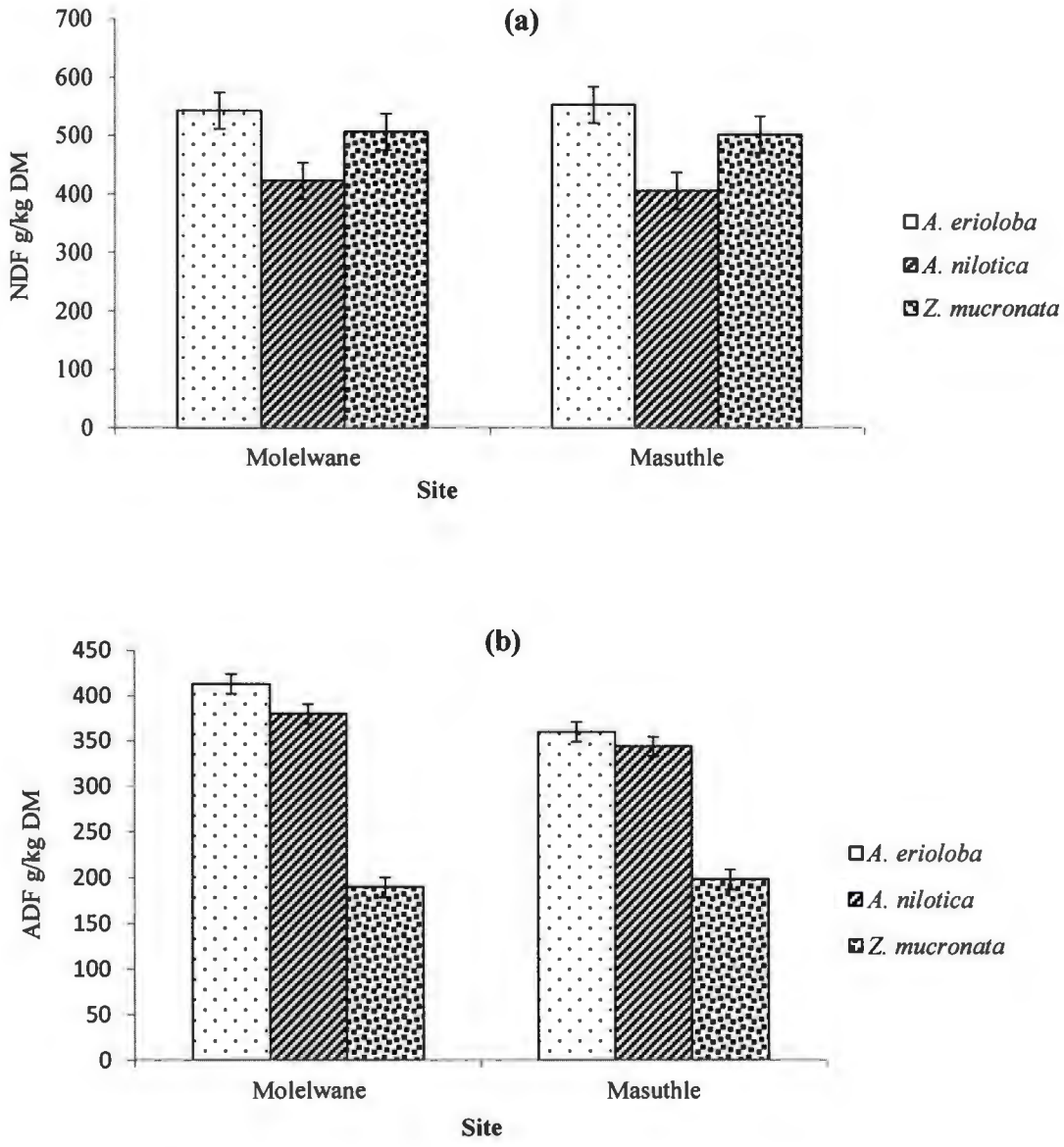


Figure 3.1. Effect of site and species interaction on NDF (a) and ADF (b) content of leaves

Figure 3.1 (b) shows that leaves of *A. erioloba* and *A. nilotica* harvested from Molelwane (413.0 g/kg DM) had higher (P >0.05) ADF contents than those harvested from Masuthle (360.0 g/kg DM). Leaves from *Z. mucronata* had the same ADF content between the two sites.

3.3.2 Minerals

The macromineral components of leaves from *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species, expressed on a DM basis, are shown in Table 3.2. Site and species did not significantly affect all macrominerals. The interaction between site and tree species was not significant for all macrominerals except sulphur (S).

Table 3.2. Macro-minerals (g/100 g DM) content in leaves of *Acacia erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from Molelwane and Masuthle

	Molelwane			Masuthle			
Parameters	[1]AE	[2]AN	[3]ZM	AE	AN	ZM	SEM
Ca	6.78	5.23	7.52	3.15	5.44	4.81	1.13
P	0.04	0.05	0.06	0.05	0.06	0.05	0.007
S	0.83^{bA}	0.38^{aA}	0.51^{abA}	0.45^{aB}	0.66^{abA}	0.41^{aA}	0.09
Na	0.02	0.03	0.03	0.02	0.03	0.03	0.005
Mg	0.28	0.19	0.28	0.16	0.24	0.21	0.03

[1]AE = *Acacia erioloba*; [2]AN = *Acacia nilotica*; [3]ZM = *Ziziphus mucronata*

[a,b] In a row, different superscripts denote significant differences (P <0.05) between tree species

[AB] Uppercase superscripts compare sites for each tree species

There was no significant difference between Ca, P, Na and Mg contents for all tree species. Leaves of *A. erioloba* harvested in Molelwane had the highest S content (0.83 g/100 g DM) compared to those in Masuthle (0.45 g/100 g DM). In both sites, leaves of *A. nilotica* and *Z. mucronata* had the same (P >0.05) S content. Table 3.3 shows the levels of significance of site and species in the content of microminerals.

Table 3.3. Statistical significance of the effects of main factors on the micromineral content of leaves from *Acacia erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from Molelwane and Masuthle

	Microminerals						
Factors	Fe	Si	Mn	Cu	Zn	Mo	Co
Site	**	**	NS	*	NS	NS	NS
Species	NS	***	***	NS	NS	*	**
Site × Species	NS	NS	NS	*	NS	NS	**

NS: $P > 0.5$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

The micromineral contents of leaves from *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species, expressed on a DM basis, are shown in Table 3.4. Leaves harvested from Molelwane had a higher Si content (308.9 mg/kg DM) than those harvested from Masuthle (220.1 mg/kg DM). In both sites, leaves from *A. erioloba* and *A. nilotica* had the same ($P > 0.05$) Si content, together with *Z. mucronata* harvested in Molelwane.

Table 3.4. Microminerals of leaves (mg/kg DM) from *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* harvested from Molelwane and Masuthle

	Molelwane			Masuthle			
Parameters	[1]AE	[2]AN	[3]ZM	AE	AN	ZM	SE
Si	237.7^{ab}	370.2^{b}	318.7^{b}	277.1^{b}	248.0^{ab}	135.2^{a}	34.39
Fe	227.5	209.4	195.0	153.0	163.4	142.6	25.13
Mn	50.3	28.8	170.7	17.5	26.8	149.8	19.16
Zn	7.56	5. 29	7.10	5.52	5.46	7.03	0.86
Cu	1.65^{a}	2.41^{a}	2.62^{a}	3.01^{a}	4.03^{b}	2.08^{a}	0.43
Co	0.10^{aB}	0.10^{aB}	0.10^{aB}	0.12^{bB}	0.11^{bB}	0.05^{aA}	0.01
Mo	0.11	0.13	0.11	0.12	0.21	0.12	0.23

[1]*Acacia erioloba*; [2]AN = *Acacia nilotica*; [3]ZM = *Ziziphus mucronata*

[a,b] In a row, within site, different superscripts denote significant differences (P <0.05) between tree species

[AB] Uppercase superscripts compare sites for each tree species

Leaves harvested from Molelwane had a higher Fe content (210.6 mg/kg DM) than those harvested from Masuthle (153.0 mg/kg DM). Leaves of *Z. mucronata* had the highest Mn content (160.3 mg/kg DM), while leaves of *A. erioloba* had the higher Mn content (33.9 mg/kg DM), and those of *A. nilotica* had the least Mn content (27.8 mg/kg DM). Leaves from all three species had similar (P >0.05) levels of Zn. Leaves of *A. erioloba* had the same (P >0.05) Cu content as leaves of *Z. mucronata* harvested across the two sites. Leaves of *A. nilotica* harvested from Molelwane had the least Cu content (2.41 mg/kg DM) than those harvested from Masuthle (4.03 mg/kg DM). Leaves of *A.*

erioloba and *A. nilotica* harvested from Masuthle (0.115 mg/kg DM) had a higher Co content than those harvested in Molelwane (0.10 mg/kg DM). Leaves of *Z. mucronata* harvested from Molelwane had a higher Co content (0.10 mg/kg DM) than those harvested from Masuthle (0.05 mg/kg DM). Leaves of *A. nilotica* had the highest Mo content (0.17 mg/kg DM), while leaves of *Acacia erioloba* had the higher Mo content (0.116 mg/kg DM), and those of *Z. mucronata* had the least Mo content (0.115 mg/kg DM).

3.3.3 Phenolics

The phenolic components (soluble phenolics (SPh) and soluble condensed tannins (SCT)) of leaves harvested from *A. erioloba*, *A. nilotica* and *Z. mucronata* are shown in Figure 3.2 (total soluble phenolics) and Table 3.5 (soluble condensed tannins). Only site had a significant effect (P <0.05) onto the SPh content of leaves from all tree species. There were significant (P <0.05) differences between tree species in terms of SCTs content, and there was a significant (P <0.05) interaction between site and tree species in terms of SCTs content of leaves.

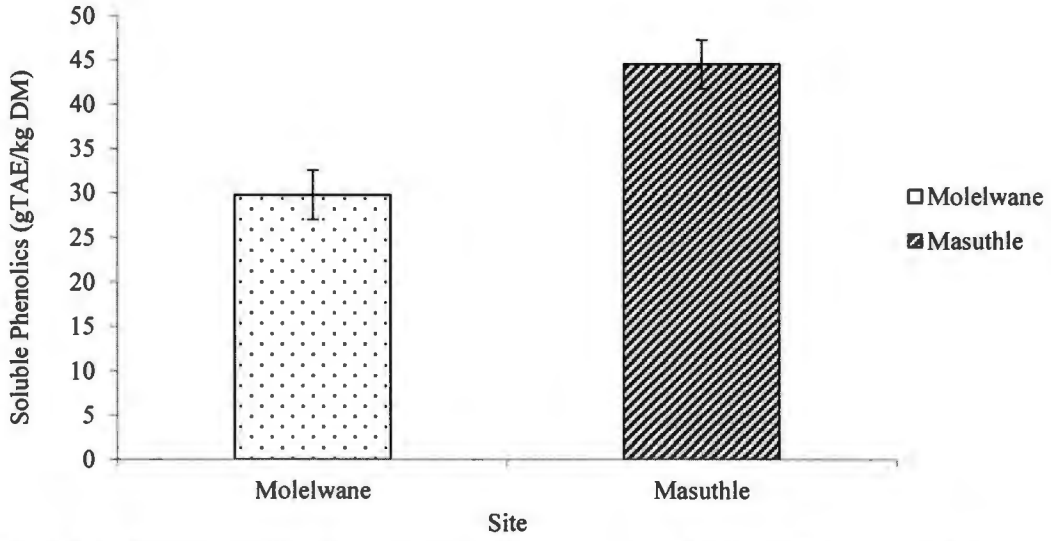


Figure 3.2. Total soluble phenolic content (g TAE/kg DM) of tree leaves harvested from two different sites

Tree leaves harvested from Masuthle had higher soluble phenolics (44.58 g TAE/kg DM) than those harvested from Molelwane (29.803 g TAE/kg DM).

Table 3.5. Soluble condensed tannin content ($AU_{550/200mg}$) of leaves from three tree species harvested from Molelwane and Masuthle

	Tree species		
Site	*A. erioloba*	*A. nilotica*	*Z. mucronata*
Molelwane	$0.12^{aA}\pm0.073$	$0.76^{bA}\pm0.076$	$0.18^{aA}\pm0.073$
Masuthle	$0.21^{aA}\pm0.073$	$0.52^{bA}\pm0.073$	$0.28^{abA}\pm0.076$

[a,b] In a row, within site, different superscripts denote significant differences (P <0.05) between tree species

[AB] Uppercase superscripts compare sites for each tree species

In Molelwane, leaves from *A. nilotica* had a higher (P <0.05) soluble condensed tannin (SCT) content than *A. erioloba* and *Z. mucronata* leaves, which did not differ (P *>0.05*). In Masuthle, *Z. mucronata* leaves had the same SCT content as those of *A. erioloba* and *A. nilotica* leaves, which significantly differed. Leaves of *A. erioloba* had the least SCT content (0.210 $AU_{550/200mg}$) compared to *A. nilotica* (0.520 $AU_{550/200mg}$). There was no significant difference in SCT content between leaves harvested from Molelwane and Masuthle for all tree species.

3.4 Discussion

Effective strategies to improve the utilisation of a feed by ruminant animals rely on the understanding of the chemical constituents that make up the diets and also the interactions of the chemical components (Barnes *et al.*, 2007). The chemical composition of leaves from *A. erioloba, A. nilotica* and *Z. mucronata* tree species has been quantified in this study as a way to define the relevance of these tree species and importance to ruminant nutrition. Ruminants such as cattle, goats and sheep have a compartmentalised stomach, which enables them to regurgitate their cud. The microbial population in the rumen also enables the fermentation of forages and the synthesis of protein. Leaves harvested from both sites were shown to have the same potential to provide dietary fibre for ruminants. Leaves of *A. nilotica* were shown to have a lower NDF content than the other tree species, which indicate that they may not be a reliable source of dietary fibre for ruminants compared to the other tree species. Although fibre is not usually a limiting nutrient for ruminants, looking at the fibre content of *A. nilotica*, it can also be utilised in places where *Z. mucronata* and *A. erioloba* are not prevalent. These findings were in line with those of Ngwa *et al.* (2000), who reported that *A. erioloba* leaves had higher concentrations of NDF and ADF than *A. nilotica* leaves. According to Norton (1994), feedstuffs with low NDF content (20 – 35%) tend

to have a higher digestibility compared to those whose NDF content is greater than 35%. The NDF content of the three tree species was higher than those reported by Norton (1994), which indicates that their digestibility would not be very high. The higher fibre content in leaves of *A. erioloba* and *Z. mucronata* could also be explained from their genetic difference because the texture of their leaves is different as already been explained in Section 2 and their high phenolic content could attribute to their high fibre content.

Barnes *et al.* (2007) explains that a high concentration of secondary plant compounds can result in high fibre values being obtained in laboratory analyses. Indeed, leaves of *A. nilotica* had the highest condensed tannins, which may explain why they have a higher NDF content than the tree species analysed by Norton (1994). This finding is also supported by Reed (1986) who stated that the high levels of tannins do increase the dietary fibre. The ADF content of leaves harvested from Masuthle was generally lower than those leaves from Molelwane, which suggests that their digestibility would be higher. With lower ADF content, the potential digestibility of leaves from *Z. mucronata* would be greater than those of *A. erioloba* and *A. nilotica*. The deficiency of protein in grasses during the cold months normally negatively affects animal production and also the higher fibrous content contributes to limited feed utilisation. Leaves of *Z. mucronata* had a higher CP content than the *Acacia* trees investigated, which highlighted that their leaves can be a valuable potential source of protein supplement for ruminant nutrition. When compared to other plants growing in the communal veldts of Botswana such as the *Acacia fleckii*, *Diospyros lycioides* and *Ziziphus mucronata* (Aganga *et al.*, 2000), *A. erioloba* ranked lowest in terms of the crude protein content scoring. However, the protein value of the leaves is also affected by secondary plant compounds, such as tannins, that may be present. Generally, leaves from Molelwane

had high calcium content than those from Masuthle. This may probably be due to the variation in soil minerals that were not determined in this study. Calcium and P are the two most plentiful minerals in the mammalian body comprising 99% and 80% respectively, in bone and teeth tissues. In most instances, the dietary mineral concentration that is adequate for ruminant requirements is not closely defined and cannot be estimated reliably from analysis of feeds (CSIRO, 2007).

The mineral content of tree leaves was higher in Molelwane than in Masuthle, which shows that animals foraging in Molelwane were less likely to suffer from mineral deficiencies. Overall, leaves of *Z. mucronata* had lower total phenolics compared to the other two species, suggesting that they might be a safer supplement unlike leaves from the *Acacia* species, which would have had a negative effect onto protein utilization due to their high phenolic content. Quantification of both total phenolics and condensed tannins is a more informative approach to foliage quality than CT alone (Waghorn, 2008). Tree leaves harvested from Masuthle had a higher concentration of phenolics than leaves from Molelwane. This may be because Masuthle is an open communal grazing land where the plants are continuously exposed to herbivores and this may increase the production of secondary compounds as an inductive defence mechanism. Indeed, plants that evolve together with herbivores have been shown to develop more elaborate defence mechanisms, such as the synthesis of secondary plant compounds, than those protected from browsing (Chaves & Escuder, 1999). Soil factors (nutrient status and pH), environmental factors (climate, rainfall, season, and temperature), animal factors and the stage of maturity can all influence the chemical composition of browse leaves (Ibrahim *et al.*, 1988). Molelwane, on the other hand, is a private fenced piece of land in which only a few goats (about 30 goats at a time) are reared. These goats only browse for very short periods of time because they are also reared on

supplemental or bought-in feeds. This could perfectly explain the low amount of phenolics in tree leaves harvested from this farm. Chaves & Escuder (1999) have indeed confirmed that tannin concentrations in plants differ in response to environmental changes. Ngwa *et al.* (2000) reported that trees growing under stressful conditions tend to produce more tannins as another way of their defence mechanism against browsing. Leaves of *A. nilotica* harvested from both sites and leaves of *Z. mucronata* harvested from Masuthle had higher levels of CTs than what, which may be useful in promoting bypass protein for ruminant animals. Reed *et al.* (1990) reported that not all foliages containing tannins are detrimental to ruminant animals but may also have beneficial effects such as increased bypass protein and the prevention or reduction of bloat incidences.

3.5 Conclusions

Leaves of *Z. mucronata*, *A. erioloba* and *A. nilotica* can be used by farmers as possible sources of protein for animals feeding on poor quality forages with low protein content, particularly during the dry months, because their CP content was greater than 12%, which grasses cannot provide to ruminants. Leaves harvested from Masuthle had a high phenolic content, which confirmed that different growth environments influence the chemical composition of browse leaves. The two sites had similar contents for most of the mineral components even though those from Molelwane were generally higher compared to the ones from Masuthle, which means that in terms of mineral nutrition, leaves harvested from Molelwane may have adequate minerals for the foraging ruminants. Although fibre is not a limiting nutrient for ruminants, all analysed tree species had a varying potential to be used as sources of the dietary fibre.

3.6 References

Aganga, A. A., Adogla-Bessa, T., Omphile, U. J., Tshireletso. K., 2000. Significance of browses in the nutrition of Tswana goats. Archivos de Zootec 49:469-480

Agri Laboratory Association of Southern Africa (AgriLASA), 1998. Feed and Plant Analysis Methods. AgriLASA, Pretoria, South Africa.

Aganga, A.A., Tsopito, C.M., Yeboah, S.O., Mokgoko, C. & Manne, S.N., 2003. Evaluation of the chemical composition of some locally available *Acacia* seeds as animal feed in Botswana. World Anim. Rev. 23, 135-136.

AOAC, 1999. Method number 976.06. *In Official Methods of Analysis of AOAC International*, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Barnes, R.F., Nelson, C.J., Moore, K.J., Collins, M., 2007. The science of grassland agriculture. 6th ed. V.2. pp. 467 – 499.

Chaves, N & Escuder, J.C., 1999. Variation of flavonoid synthesis induced by ecological factors. In principles and practices in plant ecology – Allochemical interactions. Eds. Inderjit. Dakshini, K.M.K., Foy, C.L. pp. 267 – 285. CRC Press, Boca Raton.

CSIRO, 2007. Nutrient requirements of domesticated ruminants. CSIRO Publishing. Collingwood, Australia. Pp 1-270.

Cudjoe, N. & Mlambo, V., 2014. Buffer nitrogen solubility, *in vitro* ruminal partitioning of nitrogen and *in vitro* ruminal biological activity of tannins in leaves of four fodder tree species. J. Anim. Physiol. Anim. Nutr. 98, 722 – 730.

Ibrahim, M.N.M., Tamminga, S., Zemmelink, G., 1988. Nutritive value of some commonly available ruminant feeds in Sri Lanka. In: Dixon, R.M. (Ed.), Ruminant Feeding Systems Utilizing Fibrous Agricultural Residues, 1987, Proceedings of the Seventh Annual Workshop of the Australian–Asian Fibrous

Agricultural Residues Research Network held at Chang Mai University, Thailand, June 2–4, 1987.

Lukhule, M.S. & van Ryssen, J.B.J., 2000. The chemical composition of leaves from indigenous fodder trees in South Africa. S. Afr. J. Anim. Sci. 30 (1), 72 - 73.

Makkar, H.PS., 2003. Quantification of Tannins in Tree and Shrub Foliage. A Laboratory Manual. Pp. 43 - 54.

Ngwa, A.T., Nsahlai, I.V. & Bonsi, M.L.K., 2000. The potential of legume pods as supplements to low quality roughage. J. Sci. Food Agric. 30, 107 - 108.

Norton, B. W., 1994. The nutritive value of tree legumes. In: (Ed. R. C. Gutteridge and H. M. Shelton), Forage Tree Legumes in Tropical Agriculture, CAB international , Wallingford, UK.

Norton, B. W., and D. P., Poppi. 1995. Composition and nutritional attributes of pasture legumes. In: (J. P. F. D'Mello, C. Devendra). Tropical Legumes in Animal Nutrition. CAB International, Wallingford, UK.

Perkin-Elmer, 1982. Perkin-Elmer AAS manual (Model 2389). Norwalk, Connecticut, USA.

Porter, L.J., Hrstich, L.N. & Chan, B.G., 1986. "The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin". *Phytochemistry* 1: 223-230.

Reed, J.D., 1986. Relationships among soluble phenolics, insoluble proanthocyanidins and fiber in East African browse species. J. Range Manage. 39, 5-7.

Reed, J.D., Soller, H. & Woodward, A., 1990. Fodder tree and straw diets for sheep: Intake, growth, digestibility and the effects of phenolics on nitrogen utilisation. Anim. Feed. Sci. Technol. 30, 39.

SAS, 2010. SAS Users Guide: Statistics, Version 9.3 SAS Institute, Cary, NC, USA.

Tefera, S., Mlambo, V., Dlamini, B.J., Dlamini, A.M., Koralagama, K.D.N. & Mould, F.L., 2008. Chemical composition and *in vitro* ruminal fermentation of common

tree forages in the semi-arid rangelands of Swaziland. Anim. Feed Sci. Technol. 142, 99 - 110.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583 - 3597.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production- Progress and challenges. Anim. Feed Sci. Technol. 147, 116 – 139.

4 BUFFER NITROGEN SOLUBILITY AND *IN VITRO* RUMINAL NITROGEN DEGRADABILITY OF LEAVES FROM TREES HARVESTED FROM TWO GROWTH ENVIRONMENTS

Abstract

This study presents the buffer solubility index of nitrogen (N) and the *in vitro* ruminal dry matter (DM) and N degradation from leaves of *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* harvested from Molelwane and Masuthle. Leaves of *Z. mucronata* had a higher N content than those of the *Acacia* genus. Leaves of *A. nilotica* had a higher buffer-insolubility and it was reasoned that their high tannin content were the cause. Leaves of *A. erioloba* and *Z. mucronata* had a higher fibre content and digestibility. In both sites, N in *A. nilotica* leaves had lower solubility index (SI) than in *A. erioloba* and *Z. mucronata* leaves, which did not differ (P >0.05). Leaves of *Z. mucronata* had a higher DM ruminal disappearance than those of the *Acacia* tree species, whose DM disappearance did not differ at all incubation times. Ruminal N degradability was similar across all tree species at 24 hours of incubation, however, at 12 h species differences were observed. For leaves harvested in Molelwane, there was a significant (P <0.05) positive correlation between N solubility index and *in vitro* ruminal N degradability at 4, 12, 36 and 72 hours. For leaves harvested in Masuthle, there was no significant (P >0.05) correlation between N solubility index and *in vitro* ruminal N degradability. It was concluded that buffer-soluble N cannot be used to estimate the *in vitro* N degradability of leaf substrates harvested from Masuthle, which had high phenolic levels.

4.1 Introduction

While the previous investigation revealed that leaves from *A. nilotica, A. erioloba* and *Z. mucronata* contained moderate levels of protein that could be useful to ruminants, the availability of the protein to rumen microbes remains unknown. The degradation of protein in the rumen is one of the most important factors that determine the value of protein in any feedstuff for to ruminants. The confirmed presence of secondary plant compounds in the leaves is likely to affect rumen degradability and hence utility of the assayed protein. The *in vitro* ruminal fermentation technique is generally considered as a reference method to determine ruminally degradable substrates (De Boever *et al.*, 2004). However, this technique requires the use of surgically modified animals and is also rather laborious. This has led to the development of other procedures designed to predict degradability of protein in the rumen.

One such rapid approach is to determine the solubility of protein in a buffer at 39°C. Greater buffer solubility of protein translates into high degradability in the rumen (Licitra *et al.*, 1996). If a strong relationship between buffer solubility of protein and protein degradability in the rumen is established, it means one can then rely on buffer solubility to predict rumen degradability. According to Cudjoe & Mlambo (2014), the relationship between the N solubility index and *in vitro* ruminal N degradability varied with tree species, which shows that factors that influence this relationship needs further investigation. According to Beever & Mould (2000), the nutritional quality of browse leaves largely differ depending on the type of species, soil type, seasonal variations, harvest stage as well as processing of leaves. This section, therefore, aimed at determining the buffer-soluble nitrogen and the *in vitro* ruminal nitrogen degradability of leaves from *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from two different growth environments. The study tests the hypothesis that tree species and

growth environment affect the buffer nitrogen solubility index and its association with *in vitro* ruminal nitrogen degradability.

4.2 Material and methods

4.2.1 Nitrogen solubility in buffer solution

Buffer soluble nitrogen was determined according to the modified Licitra *et al.* (1996) method in which ANKOM F57 filter bags were used for the filtration process (Cudjoe & Mlambo, 2014). ANKOM F57 filter bags (ANKOM Technology, New York) were labelled using a solvent-resistant marker. Leaf samples (0.45 – 0.5 g) were weighed using an analytical balance and then placed into the bags. An impulse heat sealer was used to seal the bags containing the samples. ANKOM Daisy^{II} incubator jars were filled with 1500 mL of a borate-phosphate and 10% sodium azide buffer solution (see Appendix 1) and set at a temperature of 39°C with agitation in an incubation chamber. After 3 hours, the jars were removed and the solution was poured out and the samples removed from the jars. Samples were squeezed to remove excess solution, by placing them in an air-drying pan before being oven-dried at 105°C for 12 h. After drying, the samples were removed from the oven and placed in a desiccant pouch to cool before weighing. Nitrogen (N) residue was determined using the standard macro-Kjeldahl method (AOAC 1999, method no. 984.13) and estimated as insoluble N content of the samples. Soluble N content was calculated as the difference between the total and insoluble N contents. Buffer solubility index was calculated as a ratio between the soluble N and the total N contents.

4.2.2 The *in vitro* ruminal DM and N degradation

The *in vitro* ruminal DM degradability of leaf samples was determined using the Daisy^{II} incubator consisting of a thermostatic chamber (39°C) with four rotating jars, and according to the ANKOM Technology Method 3 for *in vitro* true digestibility (ANKOM Technology Corp., Fairport, NY). Ground samples (2 mm) were weighed into ANKOM F57 bags (0.45 - 0.5 g), heat sealed and placed into the digestion jars. Two buffer solutions, A and B (see Appendix 2) were prepared in advance and combined at a ratio of 1:5, and according to the ANKOM Technology Method no. 3 (ANKOM, 2005), and 1600 mL of this combined buffer was then transferred to each of the jars and warmed.

A rumen inoculum was collected in the morning before feeding from a ruminally cannulated Bonsmara cow with a body weight of approximately 550 kg. The Bonsmara cow was receiving a ration that was a mixture of lucerne and blue buffalo grass. She was kept in a free stall and had free access to clean water. Rumen fluid was collected into two pre-warmed thermos flasks and quickly taken to the laboratory, where it was blended and strained through two layers of warm muslin cloths. Blending was done to dislodge microorganisms (e.g. *Fibrobacter succinogens* and *Ruminococcus flavefaciens*) that attach to fibrous substrates. Strained rumen fluid was held at 39°C under a stream of carbon dioxide gas. Four Daisy^{II} jars, each containing 1600 mL of buffer and leaf samples sealed in ANKOM F57 bags, were inoculated by adding 400 mL of the rumen inoculum to each digestion jar. Each jar was then purged with CO_2 before being covered and placed into the incubation chamber. Filter bags were withdrawn at 12, 24, and 36 hours after inoculation. Withdrawn bags were washed with cold water for 20 minutes and using the ANKOM^{2000} Fibre Analyser. The washed samples were then dried at a temperature of 105°C for 12 hours, and N determined on

the residues using the macro-Kjeldahl method (AOAC, 1999 method no. 984.13). Nitrogen degradability was eventually estimated as the loss in total N upon incubation.

The *in vitro* ruminal DM degradability was determined using the following formula:

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

Where, W1 = Bag tare weight, W2 = Sample weight, W3 = Final bag weight after *in vitro* treatment, and C1 = Blank bag correction factor (final oven-dried weight ÷ original blank bag weight).

4.2.3 Statistical analysis

4.2.3.1. Two-way analysis of variance

The buffer N solubility and *in vitro* ruminal degradability of DM and N in *A. erioloba*, *A. nilotica* and *Z. mucronata* leaves were analysed using the general linear models (GLM) procedure of SAS 9.3 (2010) for a 3 x 2 factorial treatment arrangement in a completely randomized experimental design. Main effects were tree species (*A. erioloba*, *A. nilotica* and *Z. mucronata* tree species) and growth environment (Molelwane and Masuthle). The general linear model was as follows.

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

Where, Y_{ijk} = dependant variable, μ = population mean, S_i = effect of tree species, GE_j = effect of growth environment, $(S \times GE)_{ij}$ = effect of interaction between tree species and growth environment, and E_{ijk} = random error associated with observation *ijk*,

assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P \leq 0.05$. Least squares means were compared using the probability of difference (pdiff) option in the lsmeans statement of SAS.

4.2.3.2. Correlation analysis

The strength of the linear relationships between N buffer solubility and N degradability were measured using the Pearson's correlation coefficients. The correlation procedure of SAS (2010) was employed for this analysis.

4.3 Results

Table 4.1 summarizes the statistical significance of the main factors and their interaction. The buffer soluble nitrogen indices of leaves harvested from *Acacia erioloba*, *A. nilotica* and *Z. mucronata*, expressed on an N basis, are shown in Table 4.2, together with the total N, expressed on a DM basis.

Table 4.1. Statistical significance (*P* values) of the effects of main factors and their interactions on the buffer nitrogen solubility indices of leaves from *Acacia erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from Molelwane and Masuthle

Site	Total N (g/kg DM)	Buffer Insoluble N (g/kg N)	Buffer Soluble N (g/kg N)	Solubility Index
Site	NS	NS	NS	NS
Species	*	***	***	***
Site × Species	NS	*	*	*

NS: $P > 0.5$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 4.2 indicates that the total N content of leaves harvested from the three tree species. The buffer-insoluble nitrogen (BINSN) of leaves harvested from the three species ranged from 740.0 – 771.5 g/kg N. In both sites, leaves of *A. erioloba* were shown to have the same (P >0.05) BINSN content on an N basis. There was no significant (P >0.05) difference in the BINSN content of the leaves of *A. nilotica* and *Z. mucronata*.

Table 4.2. Total nitrogen and buffer nitrogen solubility parameters in leaves from *Acacia erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from two different sites (Molelwane and Masuthle)

Parameters	Molelwane			Masuthle			
	[1]AE	[2]AN	[3]ZM	AE	AN	ZM	SEM
Total N (g/kg DM)	23.5^{ab}	20.3^{a}	29.1^{c}	24.7^{abc}	20.5^{a}	27.8^{bc}	1.27
Buffer Insoluble N (g/kg N)	753.6^{a}	884.8^{b}	740.4^{a}	813.7^{ab}	850.7^{b}	771.5^{a}	17.67
Buffer Soluble N (g/kg N)	246.4^{b}	115.2^{a}	259.6^{b}	186.3^{ab}	149.3^{a}	228.5^{b}	17.67
Solubility Index (%)	24.6^{b}	11.5^{a}	26.0^{b}	18.6^{ab}	14.9^{a}	22.9^{b}	1.77

[1]AE = *Acacia erioloba*; [2]AN = *Acacia nilotica*; [3]ZM= *Ziziphus mucronata*

[a,b] In a row, within site, different superscripts denote significant differences (P <0.05) between tree species except for total N

[a,b,c] In a row, different superscripts denote significant differences (P <0.05) between tree species

The buffer-soluble N content of *A. erioloba* leaves harvested from Molelwane (246.4 g/kg N) was higher than that of leaves harvested from Masuthle (186.3 g/kg DM). In both sites, leaves of *A. nilotica* had the same (P >0.05) buffer-soluble N content on an N basis. Leaves of *Z. mucronata* harvested from Molelwane had the same (P >0.05) BSN content on an N basis compared to those harvested from Masuthle. The solubility

index (SI) of leaves harvested from the three species ranged from 11.5 to 26.0%. There was no significant difference between *A. erioloba* and *Z. mucronata* leaves on the SI. Leaves of *A. nilotica* harvested from Molelwane had the same (P >0.05) SI as those harvested from Masuthle.

The DM and N degradability of leaves harvested from *Acacia erioloba*, *A. nilotica* and *Z. mucronata* are shown in Table 4.3 and Figures 4.1 and 4.2. There were significant (P <0.05) differences between species for all parameters. Site had an effect onto the DMD36. There was a significant interaction between site and species for the DMD36. Leaves of *Z. mucronata* had the higher DMD12 (318.43 g/kg DM) than those of *A. erioloba* and *A. nilotica*, which did not differ (P >0.05).

Table 4.3. *In vitro* ruminal DM and N degradability (g/kg DM) of leaves from *Acacia erioloba*, *A. nilotica* and *Z. mucronata* tree species

	[2]Species			
[1]Parameters	AE	AN	ZM	SEM
DMD 12	212.99[a]	197.51[a]	318.43[b]	12.67
DMD 24	281.93[a]	204.33[a]	418.16[b]	19.76
ND12	225.35[ab]	96.51[a]	261.65[b]	34.73
ND24	271.98[a]	152.14[a]	277.19[a]	37.48

[1]Parameters: DMD 12 = Dry Matter Degradability at 12 h after inoculation; DMD24 = Dry Matter Degradability at 24 h after inoculation; ND12 = Nitrogen degradability at 12 h after inoculation; ND24 = Nitrogen degradability at 24 h after inoculation

[2]Species: AE = *Acacia erioloba*; AN = *Acacia nilotica*; ZM = *Ziziphus mucronata*

[a,b] In a row, different superscripts denote significant differences (P < 0.05) between tree species

This was also true for the DM degradability at 24 h (DMD24), which ranged from 204.33 – 418.16 g/kg DM, with leaves of *Z. mucronata* having the highest DMD24 (418.16 g/kg DM). Molelwane had the least DMD36 (203.79 g/kg DM) as compared to Masuthle (309.08 g/kg DM). In Molelwane, leaves of *Z. mucronata* had the highest DMD36 (533.48 g/kg DM) followed leaves of the *A. erioloba* (213.28 g/kg DM) and *A. nilotica* (180.475 g/kg DM). In Masuthle, the highest DMD36 was found in leaves of *A. erioloba* followed by leaves of the *A. nilotica* and *Z. mucronata*, which did not differ (P >0.05).

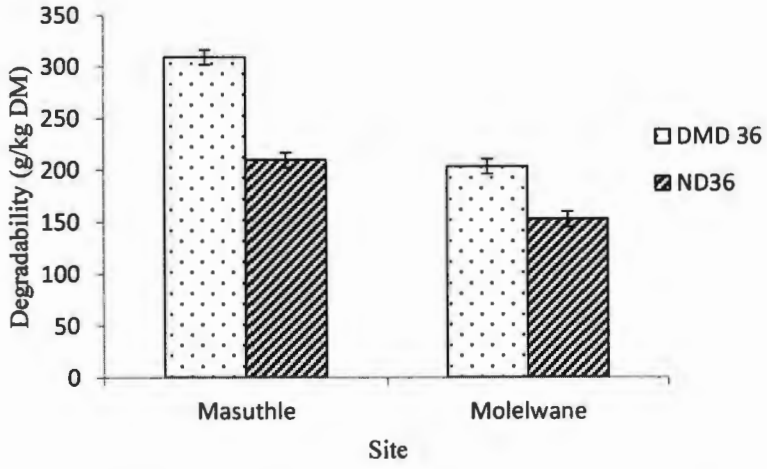


Figure 4.1. *In vitro* ruminal DM and N degradability at 36 h (g/kg DM) of leaves harvested from Masuthle and Molelwane

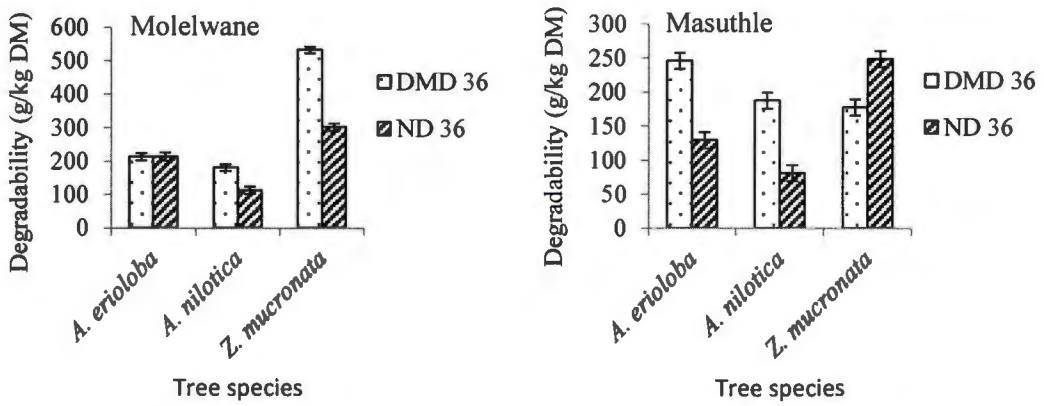


Figure 4.2. *In vitro* ruminal DM and N degradability (g/kg DM) at 36 h post-incubation of leaves harvested from Molelwane and Masuthle sites

The nitrogen degradability at 12 h (ND12) ranged from 96.51 – 261.65 g/kg DM. Leaves from *A. nilotica* had the same (P >0.05) ND12 content as those of *A. erioloba* and leaves of the *Z. mucronata* had the same (P >0.05) ND12 content as those of *A. erioloba*. *Ziziphus mucronata* tree species had a higher ND12 (261.61 g/kg N) than those of *A. nilotica* (96.51 g/kg DM). The ND24 content was the same (P >0.05) for all the three tree species. The N degradability at 36 h (ND36) ranged from 80.68 – 302.63 g/kg DM. Molelwane had the least ND36 (153.01 g/kg DM) as compared to Masuthle (209.77 g/kg DM). In Molelwane, leaves of *Z. mucronata* had the highest ND36 (302.63 g/kg DM) followed by leaves of *A. erioloba* (213.85 g/kg DM) and *A. nilotica* (112.83 g/kg DM). This was also true for leaves harvested in Masuthle, where the highest ND36 was found in leaves of *Z. mucronata* (248.88 g/kg DM) followed by leaves of *A. erioloba* 129.48 g/kg DM) and *A. nilotica* (80.68 g/kg DM).

4.3.1 Relationships between N degradability and buffer N solubility index

The Pearson's correlation coefficients for the relationship between N degradability and solubility index are shown in Table 4.4. In Molelwane, there was significant (P <0.05) relationship between the N degradability at 4, 12, 36 and 72 hours and solubility index (SI). In Masuthle, there was no significant relationship between the SI and N degradability at all-time intervals.

Table 4.4 Pearson's correlation coefficient matrix for linear relationships between Solubility index and N degradability of leaves harvested form two growth environments

		N degradability[1]						
		ND4	ND6	ND12	ND24	ND36	ND48	ND72
Molelwane	SI[2]	0.581*	0.581^{NS}	0.669*	0.517^{NS}	0.920***	0.335^{NS}	0.625*
Masuthle	SI	0.506^{NS}	0.534^{NS}	0.511^{NS}	0.535^{NS}	0.476^{NS}	0.447^{NS}	0.383^{NS}

[1]N degradability: ND4: Nitrogen degradability at 4 h after inoculation; ND6: Nitrogen degradability at 6 h after inoculation; ND12: Nitrogen degradability at 12 h after inoculation; ND24: Nitrogen degradability at 24 h after inoculation; ND36: Nitrogen degradability at 36 h after inoculation; ND48: Nitrogen degradability at 48 h after inoculation; ND72: Nitrogen degradability at 72 h after inoculation; [2]SI: Solubility Index

4.4 Discussion

A fast and efficient method to evaluate the fractionation of N in the rumen is the use of a borate-phosphate buffer N solubility. These two methods (buffer-soluble N and *in vitro* DM and N degradability) have a close association because if any feed material has high solubility; chances are that its degradability would also be high in the rumen. The

total amount of N in any leaf material indicates the total N that would be potentially available when offered to ruminants. Protein is a major deficient nutrient during the winter months therefore, a seeking of strategic supplementary sources is very crucial in order to be able to maintain growth, reproduction and production of animals.

Leaves of *Z. mucronata* had a high N content, which means that they can be a reliable source of protein during the times of low supply. The nitrogen degradation of leaves of *A. erioloba* and *Z. mucronata* were indicated to be high at 12 and 36 h than those of *A. nilotica*. This may be because *A. nilotica* leaves had the highest concentration of condensed tannins (Table 3.5), which are known to bind to proteins and making them less degradable. According to Reed *et al.* (1990), tannins have the ability to reduce fibre digestibility by binding bacterial enzymes and forming indigestible complexes. Condensed tannins have some antimicrobial effects resulting in relatively lower rumen microbial activity that leads to a depressed ruminal degradation of substrates (Reed *et al.*, 1990). The amount of protein in a diet alone cannot apparently define the quality of protein for ruminants, so the degradability of protein in the rumen is a major aspect which defines its quality. An undegradable protein feed stuff can be provided if there is a real need to increase the amount of bypass protein from the rumen, for example, leaves of *A. nilotica* can be used in high production sectors, and especially in dairy set-ups where cows are expected to produce at high levels. For high-producing ruminants, microbial protein may not be enough to meet the essential amino acid requirements. The need to meet the requirements of the rumen microbial population is very important, especially when ruminants are fed poor quality forages and high phenolic diets like leaves of *A. nilotica*.

Leaves of *A. erioloba* and *Z. mucronata* may be useful as protein supplements for high fibre and low protein diets because of their high nitrogen and solubility index, which

would increase the amount of the degradable protein in the rumen to support microbial activity. Leaves of *Z. mucronata* had the highest buffer-soluble nitrogen (BSN) and also the N degradability at 12, 24 and 36 hours, which indicated a strong relationship between the two methods mentioned above. According to Chumpawadee *et al.* (2005), highly soluble substrates tend to be highly degradable because microorganisms are able to attach readily to soluble fractions.

The buffer-soluble nitrogen has a positive and strong relationship with the N degradability at all-time intervals. In Molelwane, the correlation between SI and N degradability differed across all time intervals (4, 12, 36 and 72 hours). In Masuthle, the correlation was not significant at all the incubation time intervals, and this may be because of the higher phenolic content. This suggests that buffer-soluble N may not be used to estimate the *in vitro* ruminal N degradability of leaf substrates that have high phenolic content. According to Licitra *et al.* (1996), a higher tannin content result in increased N insolubility, and this was true for the leaves of *A. nilotica*, which were shown to have a high amount of condensed tannins and a low level of buffer solubility. Phenolics are highly soluble when compared to N. Therefore, higher phenolic content in Masuthle might have suppressed the microbial activity and consequently influencing its SI and the rumen degradability. Mlambo *et al.* (2009) suggested that the non-tannin phenolic contents in a substrate also have a negative influence onto the microbial activity, which would then interfere with its digestibility.

4.5 Conclusions

The phenolic content in leaves of all three studied tree species appeared to cause variations in buffer N solubility as well as their rumen degradability. Leaves of *A. nilotica* had the highest tannin content, which then reduced the buffer solubility and the

in vitro ruminal degradability of N. Leaves of *A. erioloba* and *Z. mucronata* had lower tannin contents, which then resulted in a higher buffer solubility of N. Growth environments were shown not to have any effect onto the *in vitro* ruminal degradability of leaf materials. Leaves of *A. nilotica* can be used to supplement high producing classes of animals such as pregnant or lactating does and ewes, while leaves of *Z. mucronata* and *A. erioloba* can be used under normal production systems or during the dry season for maintenance of animals because they had higher rumen degradability and solubility compared to *A. nilotica*. Buffer-soluble N cannot be used to estimate the *in vitro* ruminal degradability of leaf substrates harvested from Masuthle, which had high levels of phenolics.

4.6 References

ANKOM Technology, 2005. *In vitro* true digestibility using the Daisy ^{II} incubator. ANKOM Technology, Fairport, NY. USA.

AOAC, 1999. Method number 976.06. *In Official Methods of Analysis of AOAC International*, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Beever, D.E. & Mould, F.L., 2000. Forage evaluation for efficient ruminant livestock production. In: Givens, D.I., Owen, E., Axford, R.F.E., Omed, H.M. (Eds.), Forage Evaluation in Ruminant Nutrition. CAB International, pp. 15 - 42.

Chumpawadee, S., Sommart, K., Vongpralub, T., & Pattarajinda, V., 2005. *In sacco* degradation characteristics of protein feed sources in Brahman-Thai native crossbred Steers. Walailak J. Sci. Tech. 2, 219 – 229.

Cudjoe, N. & Mlambo, V., 2014. Buffer nitrogen solubility, *in vitro* ruminal partitioning of nitrogen and *in vitro* ruminal biological activity of tannins in leaves of four fodder tree species. J. Anim. Physiol. Anim. Nutr. 98, 722 - 730.

De Boever, J.L., Vanacker, J.M., Fiems, L.O., De Brabander, D.L., 2004. Rumen degradation characteristics and protein value of grassland products and their prediction by laboratory measurements and NIRS. Anim. Feed Sci. Technol. 116, 53 – 66.

Licitra, G., Hernandez, T.M. & Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347 - 358.

Mlambo, V., Mould, F. L., Smith, T., Owen, E., Sikosana, J. L. N. & Mueller-Harvey, I., 2009: *In vitro* biological activity of tannins from *Acacia* and other tree fruits: correlations with colorimetric and gravimetric phenolic assays. S. Afr. J. Anim. Sci. 39, 131 - 143.

Reed, J.D., Soller, H. & Woodward, A., 1990. Fodder tree and straw diets for sheep: Intake, growth, digestibility and the effects of phenolics on nitrogen utilisation. Anim. Feed. Sci. Technol. 30, 39.

SAS, 2010. SAS Users Guide: Statistics, Version 9.3 SAS Institute, Cary, NC, USA.

5 *IN VITRO* RUMINAL GAS PRODUCTION AS A DIAGNOSTIC TOOL FOR THE BIOLOGICAL ACTIVITY OF CONDENSED TANNINS IN TREE LEAVES

Abstract

The objective of the current study was to investigate the impact of tannins on *in vitro* ruminal fermentation parameters of tree leaves. *Acacia erioloba*, *A. nilotica* and *Z. mucronata* leaves harvested from two different growth environments were fermented with rumen fluid with or without a tannin-binding compound, polyethylene glycol (PEG), using a fully automatic ANKOM^{RF} gas production system. The inclusion of PEG consistently increased the cumulative gas production (PEG effect) from leaves of all the three tree species. However, PEG effect was lower in leaves harvested from Molelwane farm when compared to those harvested from Masuthle communal grazing land. The largest PEG effect was seen in leaves of *A. nilotica* (448%) harvested from Masuthle after 48 h of incubation. While, the least PEG effect was found in leaves of *Z. mucronata* (19%) harvested from Molelwane after 48 h of incubation. Gas production from the immediate soluble fraction (*a*) was higher in Z. *mucronata* leaves (with and without PEG) followed by leaves from the two *Acacia* trees, which did not differ (P >0.05). The inclusion of PEG increased gas production from the slowly fermentable fraction (*b*) for all three tree species. The rate of gas production from the insoluble fraction (*b*), known as *c* fraction, was higher for leaves treated with PEG (4.62%/hour) than those without PEG treatment (3.79%/hour). The *in vitro* ruminal organic matter degradability (iOMD) increased with the PEG inclusion for all the three tree species. *Ziziphus mucronata* leaves, harvested from both sites, had higher ($P < 0.05$) iOMD than those of *A. nilotica* and *A. erioloba*, which did not differ ($P > 0.05$). However, PEG inclusion caused a reduction of the partitioning factors (PF) for all the three tree

species. In both sites, soluble phenolics had no relationship ($P < 0.05$) with the PEG effect in leaves of all the three tree species. However, condensed tannin concentration was shown to be positively correlated with PEG effect at 24 and 48 hours post-incubation in Molelwane and 24 and 36 hours post-incubation in Masuthle. It was therefore concluded that the leaves of *A. nilotica* were rich in tannin content would depress rumen microbial activity and therefore should be used with caution or at least with a tannin-inactivating agent when used to supplement fibrous diets, which are low in protein. Leaves of *Z. mucronata*, which had the least PEG effect but high CP content can then be safely used with fibrous basal diets because it will be a good source of rumen degradable protein.

5.1 Introduction

The evaluation of ruminant feeds by use of the *in vitro* ruminal gas production system has been one of the major interests in the past years (Getachew *et al.*, 1998). This technique describes the kinetics of feed ruminal fermentation, it also estimates the degradation rate, and the extent of solubility and insolubility of food fractions because it monitors gas production rates at determined time intervals (Getachew *et al.*, 1998). Gas production can also be measured using other techniques, such as the reading pressure technique. The pressure technique is, however, time-consuming and requires much physical efforts, hence the development of an automated gas production technique (Mauricio *et al.* 1999). The gas production system measures accumulated gas in the headspace of closed bottles. This system is aimed at determining the biological activity of tannins in the rumen through gas production.

Browse leaves have a potential in being used as protein supplements for poor quality forages with low protein content. Their use could, however, be been hindered by

condensed tannins that are found to interfere with the utilisation and metabolism of protein by rumen microbial populations (Mlambo *et al.*, 2009). The influence of these antinutritional factors on the nutritive value of forages depends on factors like their concentration, molecular weight and their chemical and biochemical properties (Cornou *et al.*, 2013). Tannins bind to proteins and carbohydrates and form complexes that are not available or that are not easily broken down by the rumen microbial population (Makkar, 2003). The tendency to bind to proteins increases bypass protein into the small intestines but this characteristic is not useful where feeding conditions require protein to be rumen degradable so as to support a vibrant microbial population necessary for the efficient utilization of fibrous diets. Leaf concentration of tannins alone is not directly proportional to biological activity since the latter is also affected by molecular weight and other chemical characteristics of tannins. Tannin-binding polyethylene glycol (PEG) deactivates tannins, can be used to determine how microorganism reacts to feed substrates containing tannins (Cornou *et al.*, 2013). The objective of the current study was, therefore, to determine the *in vitro* ruminal biological activity of tannins in leaves from *A. erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from two different growth environments. The alternative hypothesis tested that the three tree species and growth environment affect the *in vitro* ruminal gas production.

5.2 Materials and methods

5.2.1 The *in vitro* ruminal gas production as a tannin bioassay

The effect of tannins onto the *in vitro* ruminal microbial fermentation was evaluated with the aid of a tannin-binding compound, polyethylene glycol (PEG) (M_r 4400, Associated chemical enterprises (LTD)), using the ANKOM^{RF} gas production system

(ANKOM Technology Corporation, Fairport, NY) in the Biology laboratory of the North-West University. A total of 68 milled leaf samples (1 g each) were weighed into individual ANKOM^{RF} bottles. The ANKOM buffer solution (90 mL per bottle) without PEG was added to 38 modules (including two blanks for each treatment). To the remaining 38 modules, PEG-dissolved (400 mg PEG/90 mL buffer) buffer solution was added. The bottles were sealed with modules equipped with pressure sensors and transferred into an incubator set at 39°C for 12 h prior to inoculation with the rumen fluid. Rumen fluid was collected from the same cannulated Bonsmara cow as previously described for the *in vitro* ruminal DM and N degradability and processed in the same way. The processed rumen fluid (25 mL) was used to inoculate each bottle and incubated at 39°C, simulating the temperature of the rumen. Headspace gas pressure was automatically measured at 10 min intervals until 96 h after inoculation. The gas pressure measured was then converted into moles of gas produced using the *'ideal' gas law* and then converted to millilitres (mL) of gas produced using the *Avogadro's law*. Cumulative gas production parameters were estimated by fitting data into the Ørskov & McDonald (1979) non-linear model:

$$y = a + b\left(1 - e^{-c(t-lt)}\right),$$

Where, y = gas produced at time t; a = the gas production from the immediately soluble fraction (mL); b = the gas production from the slowly soluble fraction (mL); c = the gas production rate constant for the insoluble fraction, b; t = incubation time (h); l = lag time (h). Effective gas production (Egas) was calculated, after assuming a 2% per hour outflow rate of solids. According to the equation: $Egas = a + \frac{bc}{k+c}$, where k is the assumed solid outflow rate of 2% per hour and a, b, and c are the constants from the

Ørskov & McDonald (1979) equation. Potential gas production (Pgas) was calculated as the summation of fractions *a* and *b*.

5.2.2 Estimation of the degradable substrate

Fermentation residues were recovered by filtration through sintered glass crucibles (100 – 160 µm porosity, Pyrex, Stone, UK) under vacuum to determine the end-point *in vitro* ruminal organic matter degradability. These fermentation residues were then dried at 105°C for 12 h and incinerated in a muffle furnace at 600°C overnight. A measure of fermentation efficiency, partitioning factor (mL/mg OM), was calculated as a ratio of the cumulative gas production (mL/g OM) and the *in vitro* ruminal organic matter degradability (mg) at 96 h.

5.2.3 Statistical analyses

5.2.3.1. Analysis of variance

The *in vitro* ruminal gas production parameters were analysed using the general linear models (GLM) procedure of SAS (2010). Data were analysed based on a three-way factorial treatment design within a completely randomised experimental design. Main effects were the tree species (*A. erioloba*, *A. nilotica* and *Ziziphus mucronata*), growth environment (Molelwane and Masuthle), and PEG (0 and 400 mg/sample). The linear model was as follows.

$$Y_{ijkl} = \mu + S_i + GE_j + PEG_k + (S \times GE)_{ij} + (S \times PEG)_{ik} + (GE \times PEG)_{jk} + (S \times GE \times PEG)_{ijk} + E_{ijkl}$$

Where, Y_{ijkl} = dependant variable (cumulative gas volume, *a*, *b*, *c*, *lag time*, *Egas* and *Pgas*) , μ = population mean, S_i = effect of tree species, GE_j = effect of growth environment, PEG_K = effect of polyethylene glycol, $(S \times GE)_{ij}$ = effect of interaction between tree species and growth environment, $(S \times PEG)_{ik}$ = effect of interaction

between tree species and polyethylene glycol, $(GE \times PEG)_{jk}$ = effect of interaction between growth environment and polyethylene glycol, $(S \times GE \times PEG)_{ijk}$ = effect of interaction between tree species, growth environment and polyethylene glycol, and E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P \leq 0.05$. Least squares means were compared using the probability of difference (pdiff) option in the lsmeans statement of SAS.

5.2.3.2. Correlation analyses

The strength of linear relationships between soluble phenolics, condensed tannins and biological effect of condensed tannins (PEG effect) were measured using the Pearson's correlation coefficients. The correlation procedure of SAS (2010) was used.

5.3 Results

5.3.1 Tannin bioassay

Table 5.1 below shows the statistical significance of the main factors and their interactions on cumulative gas production. The effect of PEG on *in vitro* ruminal cumulative gas production, expressed on an OM basis, is presented in Table 5.2. The PEG effect on cumulative gas at 12 h (cumgas12) ranged from 32 – 163 %.

Table 5.1. Level of significance of the effects of main factors on the *in vitro* ruminal cumulative gas production from *Acacia erioloba*, *A. nilotica* and *Z. mucronata* leaves harvested from Molelwane and Masuthle

Factors	[1]Cumgas12	[2]Cumgas24	[3]Cumgas36	[4]Cumgas48
Site	NS	NS	NS	NS
Species	***	***	***	***
PEG	***	***	***	***
Site × Species	*	*	*	**
Site × PEG	NS	NS	NS	NS
Species × PEG	NS	NS	NS	NS
Site × Species × PEG	NS	NS	NS	NS

[1]Cumgas12: Cumulative gas at 12 h; [2]Cumgas24: Cumulative gas at 24 h; [3]Cumgas36: cumulative gas at 36 h; [4]Cumgas48: cumulative gas at 48 h

NS: $P > 0.5$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

In Molelwane, Z. *mucronata* leaves had the highest PEG effect (56%) as measured by an increase in the cumulative gas production followed by *A. erioloba* (51 %) and *A. nilotica* (32%) leaves. In Masuthle, *A. nilotica* leaves had the highest PEG effect (163 %) followed by the *A. erioloba* (102%) and *Z. mucronata* (40%) leaves.

Table 5.2. Effect of polyethylene glycol on *in vitro* ruminal cumulative gas production (mL/g OM) from leaves of *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* tree species harvested in Molelwane and Masuthle

[1]Parameters	[2]Polyethylene glycol	Molelwane			Masuthle			SEM
		[3]AE	[4]AN	[5]ZM	AE	AN	ZM	
Cumgas12	-PEG	13.06^{aA}	12.22^{aA}	20.03^{aA}	12.93^{aA}	4.85^{aA}	21.25^{bA}	2.5
	+PEG	19.68^{aA}	16.17^{aA}	31.34^{bB}	26.10^{bB}	12.75^{aA}	29.82^{bA}	
	PEG effect (%)	51	32	56	102	163	40	
Cumgas24	-PEG	20.77^{aA}	16.31^{aA}	34.38^{bA}	20.33^{bA}	5.94^{aA}	34.48^{bA}	3.2
	+PEG	28.08^{aA}	21.03^{aA}	49.26^{bB}	36.84^{bB}	17.43^{aA}	46.17^{bB}	
	PEG effect (%)	35	29	43	81	193	34	
Cumgas36	-PEG	24.62^{aA}	17.79^{aA}	45.85^{bA}	23.74^{bA}	5.64^{aA}	42.14^{cA}	3.6
	+PEG	31.12^{aA}	23.83^{aA}	60.17^{bA}	41.48^{bB}	21.37^{aB}	53.37^{bA}	
	PEG effect (%)	26	34	31	75	279	27	
Cumgas48	-PEG	25.90^{aA}	18.73^{aA}	56.49^{bA}	24.81^{bA}	4.62^{aA}	48.97^{cA}	3.8
	+PEG	33.21^{aA}	27.55^{aA}	67.44^{bA}	46.11^{bA}	25.30^{aA}	61.00^{bA}	
	PEG effect (%)	28	47	19	86	448	25	

[1]Parameters: Cumgas (12, 24, 36 & 48) = cumulative gas pressure post-inoculation per time interval

[2]Polyethylene glycol: -PEG = inoculation without PEG; +PEG = inoculation with PEG

[3]AE = *Acacia erioloba*; [4]AN = *Acacia nilotica*; [5]ZM = *Ziziphus mucronata*

[a,b, c] In a row, different superscripts denote significant differences ($P< 0.05$) between tree species within site

[AB] Uppercase superscripts compare –PEG and +PEG for each tree species

The overall PEG effect on cumgas24 ranged from 29 – 193 %. The PEG effect on cumgas24 was highest for *Z. mucronata* (43%) followed by the *A. erioloba* (35%) and *A. nilotica* (29%) leaves harvested in Molelwane. In Masuthle, *A. nilotica* had the highest PEG effect followed by *A. erioloba* (81%) and *Z. mucronata* (34%). The PEG

effect on cumgas36 ranged from 26 – 279% while for cumgas48, it ranged from 19 – 448%. The PEG effect on cumgas36 and cumgas48 was highest for the *Z. mucronata*, followed by *A. erioloba* and the least was on *A. nilotica* in the two harvesting sites. Table 5.3 shows the levels of significance of site and species on *in vitro* ruminal gas production parameters (*a, b, c, lag phase, Pgas* and *Egas*).

Table 5.3. Statistical significance of the effects of main factors on the *in vitro* ruminal gas production parameters of leaves from *Acacia erioloba*, *A. nilotica* and *Z. mucronata* tree species harvested from Molelwane and Masuthle

	Parameters[1]					
Factors	*a*	*B*	*c*	*Lag phase*	*Pgas*	*Egas*
Site	NS	NS	***	NS	NS	NS
Species	***	***	***	***	***	***
PEG	*	***	***	NS	***	***
Site × Species	NS	*	***	NS	*	*
Site × PEG	NS	***	NS	NS	***	NS
Species × PEG	**	***	NS	NS	***	NS
Site × Species × PEG	NS	NS	NS	NS	NS	NS

[1]Parameters: *a*: the immediate fermentable fraction; *b*: the slowly fermentable fraction, *c*: fermentation rate of fraction *b*; Pgas: potential gas production; Egas: effective gas production
NS: $P > 0.5$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

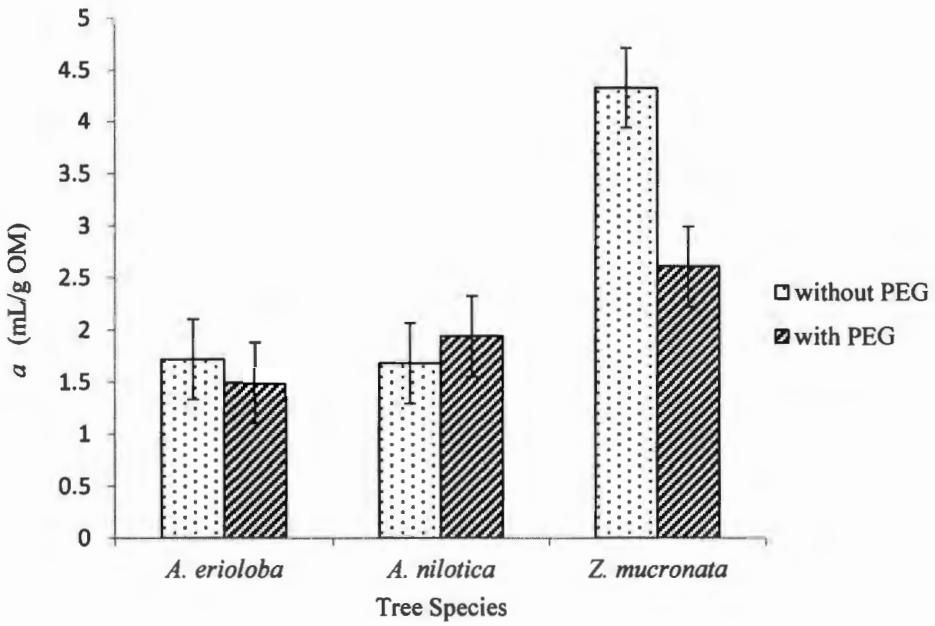


Figure 5.1. The effect of species by PEG interaction on gas (mL/g OM) produced from the immediately fermentable fraction (*a)*

Figure 5.1 shows that polyethylene glycol treatment did not affect (P >0.05) the amount of gas produced from the immediately soluble fractions of leaves of *A. erioloba* and *A. nilotica*. Leaves of *Z. mucronata* not treated with PEG had the highest gas produced from the immediately soluble fraction *a* (4.33 mL/g OM) than those treated with PEG (2.61 mL/g OM). Leaves of *A. nilotica* tree species had the same gas produced from the immediately soluble fraction *a* as was the *Z. mucronata* and *A. erioloba* when treated with PEG. However, *Z. mucronata* had a higher gas produced from the immediate soluble fraction *a* without PEG treatment than the leaves of *A. erioloba* and *A. nilotica*, which did not differ (P > 0.05).

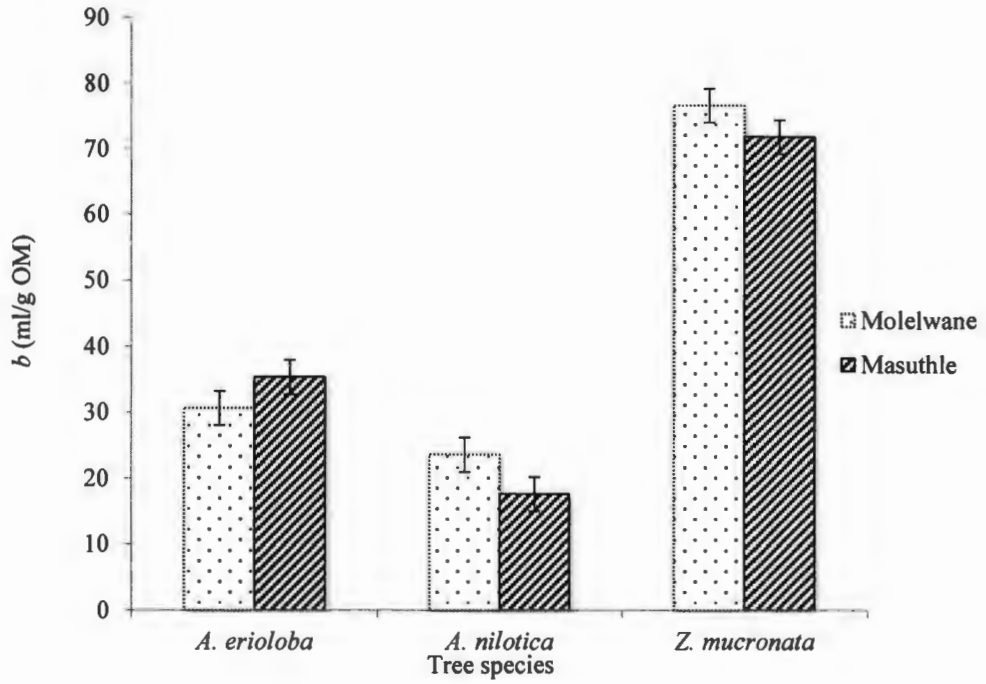


Figure 5.2. The effect of species by site interaction on gas (mL/g OM) produced from the slowly fermentable fraction (*b)*

Leaves of *A. erioloba* and *Z. mucronata* from both sites had the same gas produced from the slowly fermentable fraction *b*. Leaves of *A. nilotica* harvested from Molelwane had more gas produced from the slowly fermentable fraction *b* (17.81 mL/g OM) than those harvested from Masuthle (3.86 mL/g OM) (Figure 5.2).

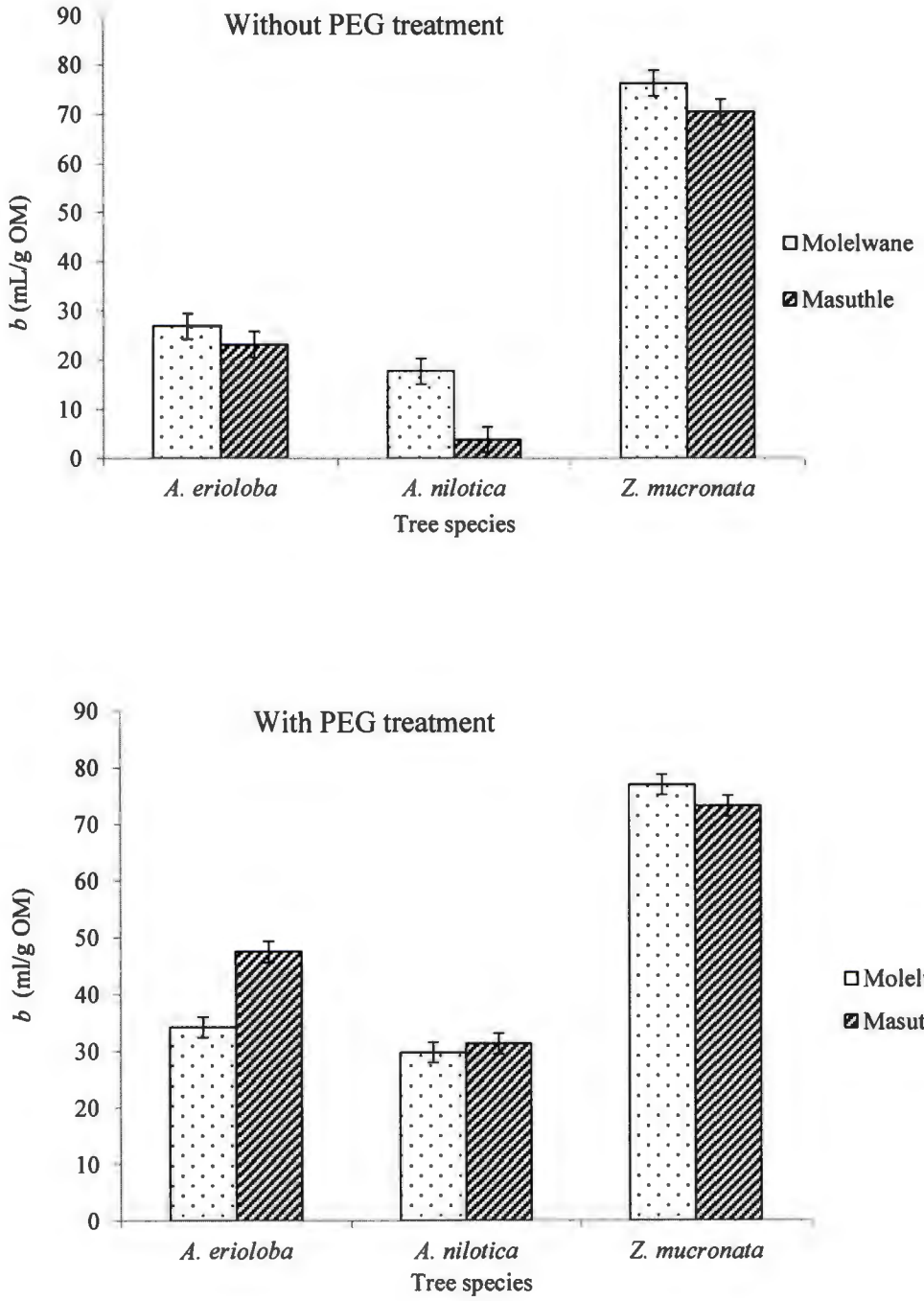


Figure 5.3. The effect of species by site interaction on gas produced (mL/g OM) from the slowly fermentable fraction *b* without polyethylene glycol (PEG) treatment and with PEG treatment

Without PEG treatment, for both sites, leaves of *A. erioloba* had the same gas production from the slowly fermentable fraction, while leaves of *A. nilotica* and *Z. mucronata* harvested from Molelwane had higher gas produced from the slowly

fermentable fraction without PEG treatment than those in Masuthle. Molelwane had higher gas produced from the slowly fermentable fraction without PEG treatment than Masuthle but the two sites had the same gas produced from the slowly fermentable fraction for only the *A. erioloba* tree species. With PEG treatment, leaves of *A. erioloba* from Molelwane had the least gas produced from the slowly fermentable fraction when compared to those harvested from Masuthle. Leaves of *A. nilotica* and *Z. mucronata* had the same gas produced from the slowly fermentable fraction in both sites (Figure 5.3).

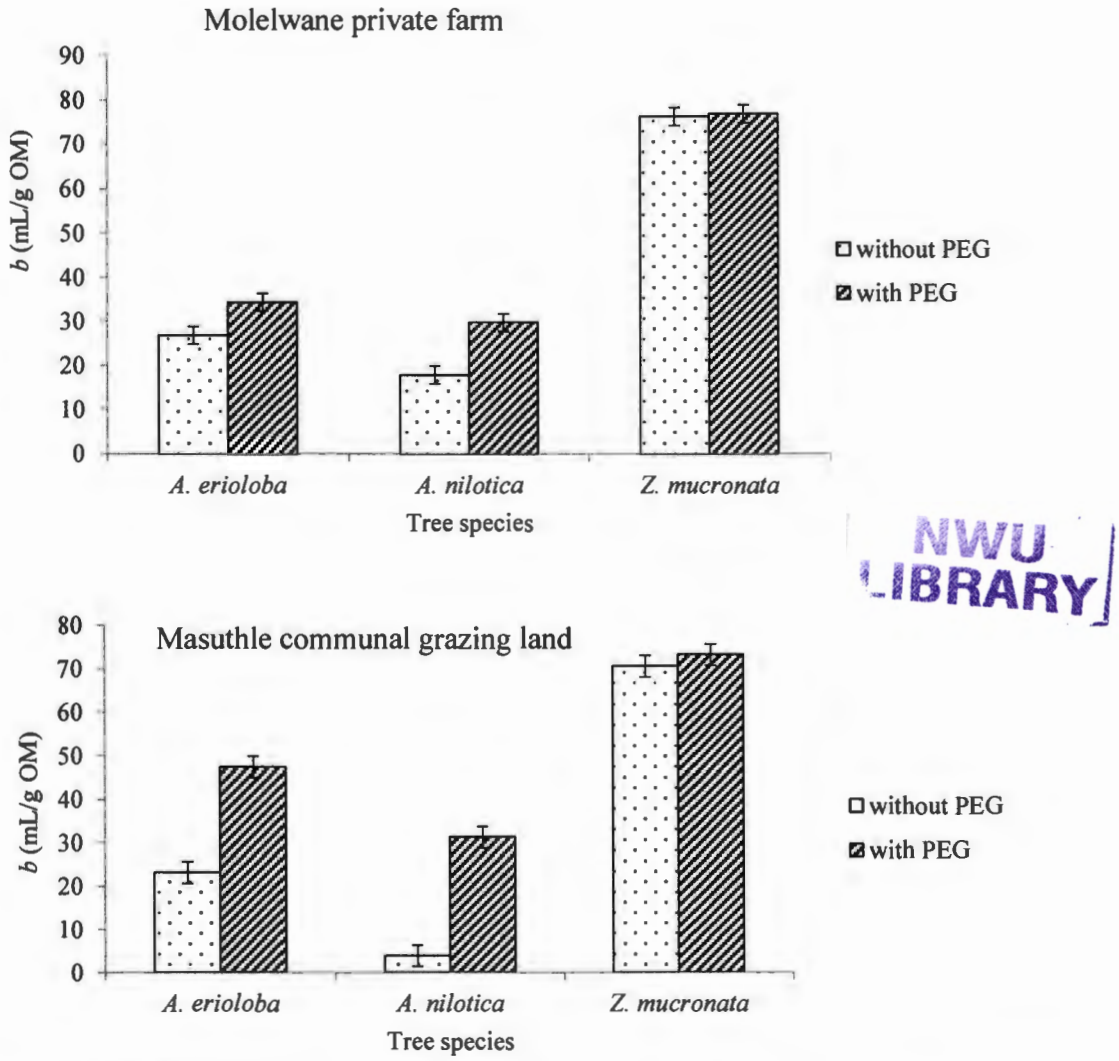


Figure 5.4. The effect of species by PEG interaction on gas produced (mL/g OM) from the slowly fermentable fraction *b* from Molelwane (a) and Masuthle (b)

In Molelwane farm, leaves of *A. erioloba* and *A. nilotica* treated with PEG had the higher gas production from the slowly fermentable fraction than those without PEG treatment. There was no significant (P >0.05) difference between the leaves of *Z. mucronata* with and without PEG treatment. With PEG treatment, leaves of *Z. mucronata* had the higher gas produced from the slowly fermentable fraction than those of *A. erioloba* and *A. nilotica*, which did not differ. Without PEG treatment, the *Z. mucronata* leaves had the highest gas production from the slowly fermentable fraction (77.9 mL/g OM), followed by *A. erioloba* (54.2 mL/g OM) and the least was from *A. nilotica* (12.5 mL/g OM) (Figure 5.4).

In Masuthle, the PEG-treated *A. erioloba* and *A. nilotica* leaves had higher gas produced from the slowly fermentable fraction than the untreated ones. There was no PEG effect (P >0.05) on *Z. mucronata* leaves. Without PEG treatment, the *Z. mucronata* leaves (70.6 mL/g OM) had the highest gas produced from the slowly fermentable fraction, followed by the *A. erioloba* (23.2 mL/g OM) and the *A. nilotica* (3.9 mL/g OM) leaves. The same trend was also observed for the PEG-treated leaves in Masuthle (Figure 5.4).

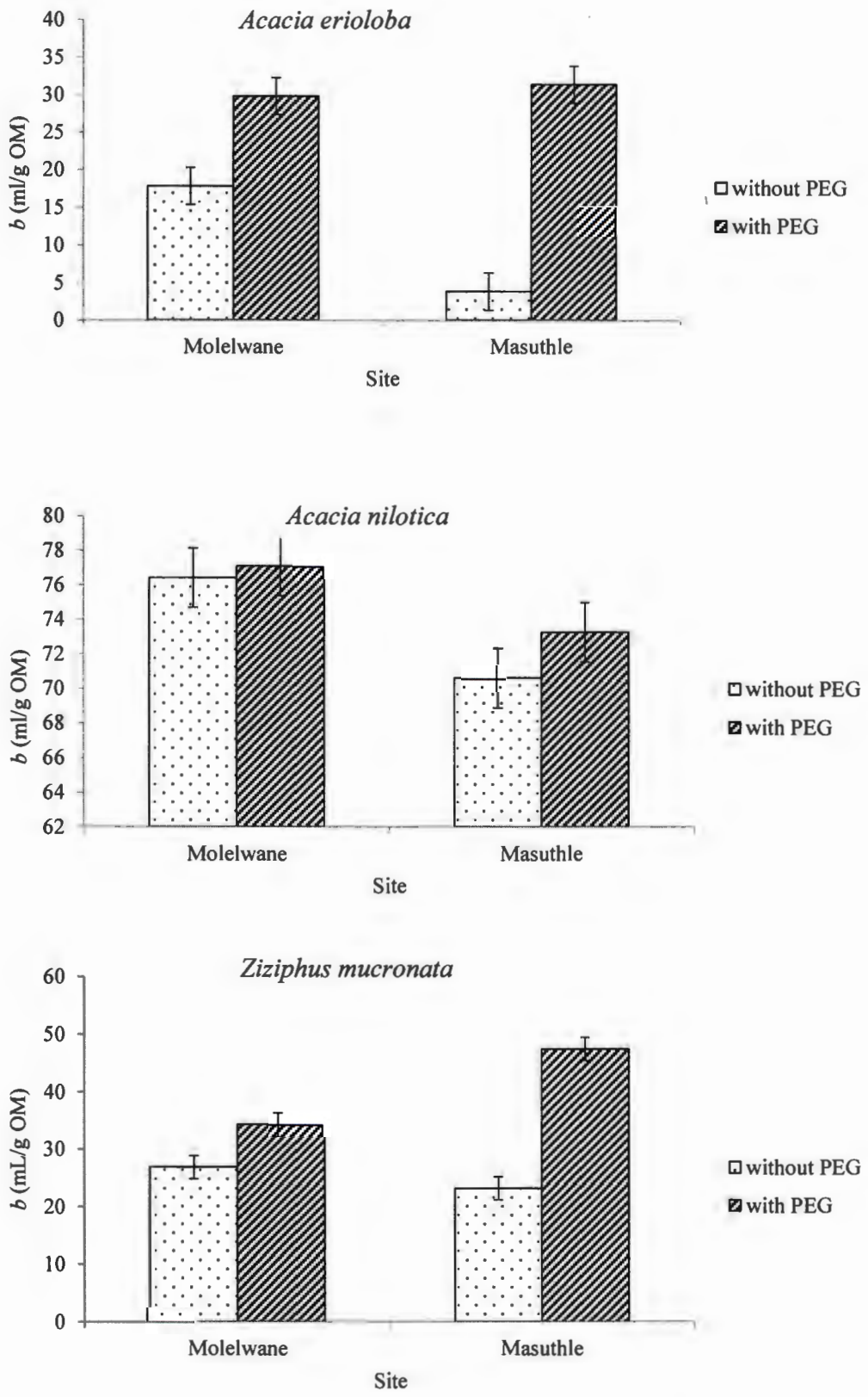


Figure 5.5. The effect of site by PEG interaction on gas production (mL/g OM) from the slowly fermentable fraction *b* in *A. erioloba*, *A. nilotica* and *Z. mucronata* leaves

Figure 5.5 shows that in both sites, the PEG-treatment increased gas production from the slowly fermentable fraction of *A. erioloba* leaves. There was no significant difference between the leaves of *A. erioloba* treated with PEG in both sites. However, leaves of *A. erioloba* harvested from Molelwane had the higher gas produced from the slowly fermentable fraction without PEG treatment than those harvested from Masuthle. There was no significant difference between the leaves of *A. nilotica* harvested from both sites. Regardless of PEG-treatment, the *A. nilotica* leaves harvested from Molelwane had the higher gas production from the slowly fermentable fraction than those harvested from Masuthle. In both sites, the PEG-treated *Z. mucronata* leaves had the highest gas production from the slowly fermentable fraction. There was no significant difference between untreated *Z. mucronata* leaves in terms of gas produced from the slowly fermentable fraction. In Masuthle, the PEG-treated *Z. mucronata* leaves produced more gas from the slowly fermentable fraction than those harvested from Molelwane. The gas produced from the *lag time* was higher for leaves treated with PEG (4.62 %/hour) than those without PEG treatment (3.79%/hour).

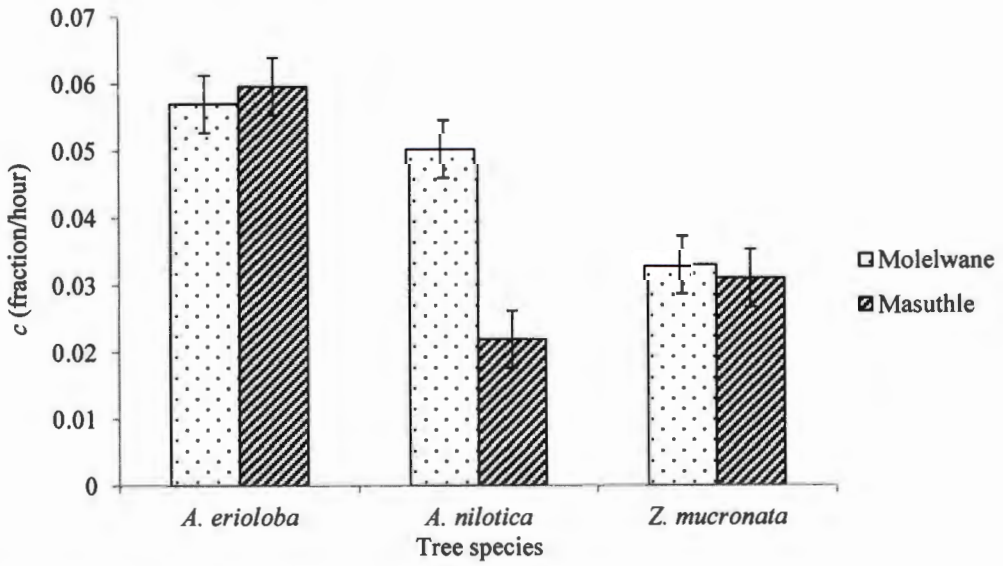


Figure 5.6. The effect of species by site interaction on the rate of gas production (*c*) from the insoluble fraction (*b*)

Figure 5.6 shows that regardless of PEG-treatment, the *A. erioloba* and *Z. mucronata* leaves had the same (P >0.05) gas production rate constant. The untreated *A. nilotica* leaves had a higher (5.03 %/h) gas production rate constant compared to the PEG-treated leaves (2.19 %/h). In Molelwane, leaves of *A. erioloba* had the same gas production rate constant as those of *A. nilotica*, which were both higher than the leaves of *Z. mucronata*. In Masuthle, *A. erioloba* leaves (5.96 %/h) had the highest gas production rate constant, followed by the *Z. mucronata* (3.06 %/h) and *A. nilotica* (2.19 %/h) leaves.

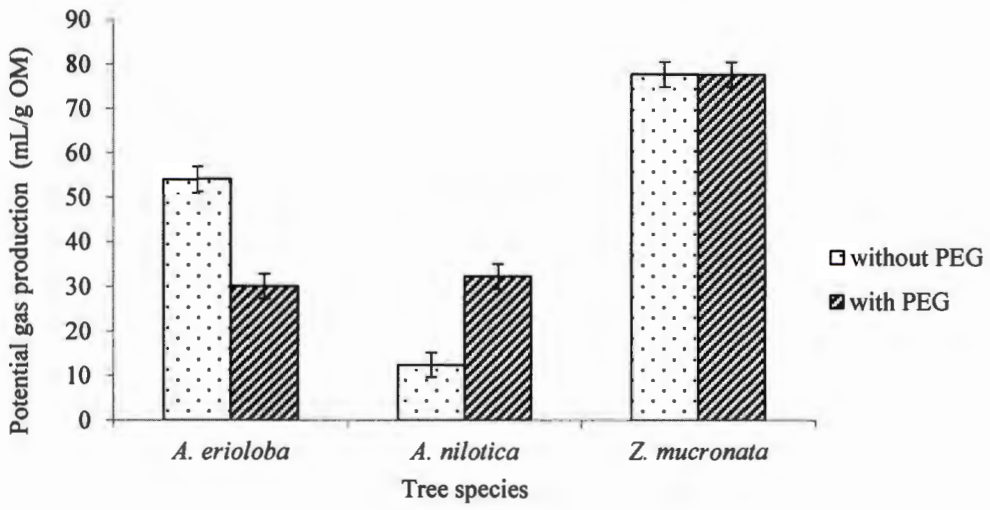


Figure 5.7. The effect of species by PEG interaction on potential gas production (mL/g OM) from tree leaves

Figure 5.7 indicates that the PEG-treatment did not affect (P >0.05) the potential gas (Pgas) production from the *Z. mucronata* leaves. The PEG-treated (19.72 mL/g OM) *A. nilotica* leaves had a higher Pgas than those without PEG treatment (5.31 mL/g OM). Leaves of *A. erioloba* had the higher Pgas without PEG treatment (54.21 mL/g OM) than when treated with PEG (30.13 mL/g OM).

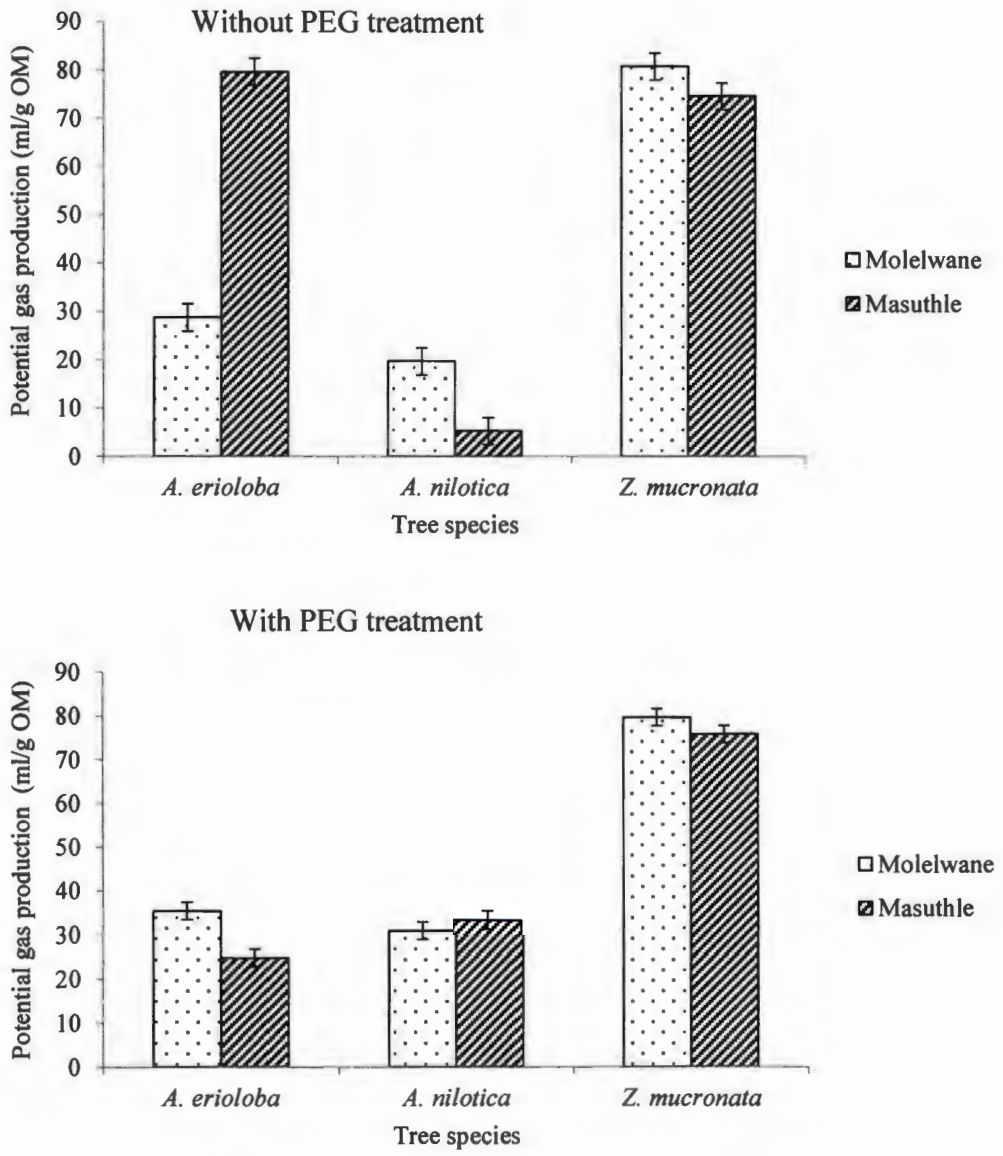


Figure 5.8. The effect of species by site interaction on potential gas production (mL/g OM) without and with PEG treatment

Figure 5.8 shows that the untreated leaves of *A. nilotica* and *Z. mucronata* harvested from Molelwane had higher Pgas values than those from Masuthle. Leaves of *A. erioloba* harvested from Molelwane had a lower Pgas (28.72 mL/g OM) compared to those harvested from Masuthle (79.69 mL/g OM). In Molelwane, leaves of *Z. mucronata* had the highest Pgas (80.93 mL/g OM) followed by the *A. erioloba* (28.72

mL/g OM) and *A. nilotica* leaves (19.72 mL/g OM), while in Masuthle, the untreated leaves of *A. erioloba* had the highest Pgas (79.69 mL/g OM) followed by the *Z. mucronata* (74.76 mL/g OM) and *A. nilotica* (5.31 mL/g OM) leaves. In both sites, leaves of *Z. mucronata* and *A. nilotica* had similar (P >0.05) Pgas values, while those of *A. erioloba* from Molelwane were higher (35.45 mL/g OM) than those from Masuthle (24.8 mL/g OM). In Molelwane, leaves of *Z. mucronata* had the highest Pgas (79.69 mL/g OM), followed by leaves of the *A. erioloba* (35.45 mL/g OM) and the least was from the *A. nilotica* (30.69 mL/g OM) leaves. In Masuthle, the least Pgas content was highest in the *Z. mucronata* leaves (75.89 mL/g OM), followed by the *A. nilotica* (33.67 mL/g OM) and the *A. erioloba* (24.8 mL/g OM).

In Molelwane, the PEG-treated leaves of *A. erioloba* and *A. nilotica* had higher Pgas levels than the untreated leaves. However, the PEG-treated leaves of *Z. mucronata* had a lower Pgas than that of the untreated leaves. In Molelwane, the untreated leaves of *Z. mucronata* had the highest Pgas produced, followed by leaves of the *A. erioloba* and *A. nilotica*. In Masuthle, the untreated leaves of *A. erioloba* had higher Pgas values compared to those treated with PEG. Leaves of *A. nilotica* treated with PEG had the higher Pgas produced than those without PEG treatment. PEG treatment on the leaves of *Z. mucronata* did not affect the Pgas.

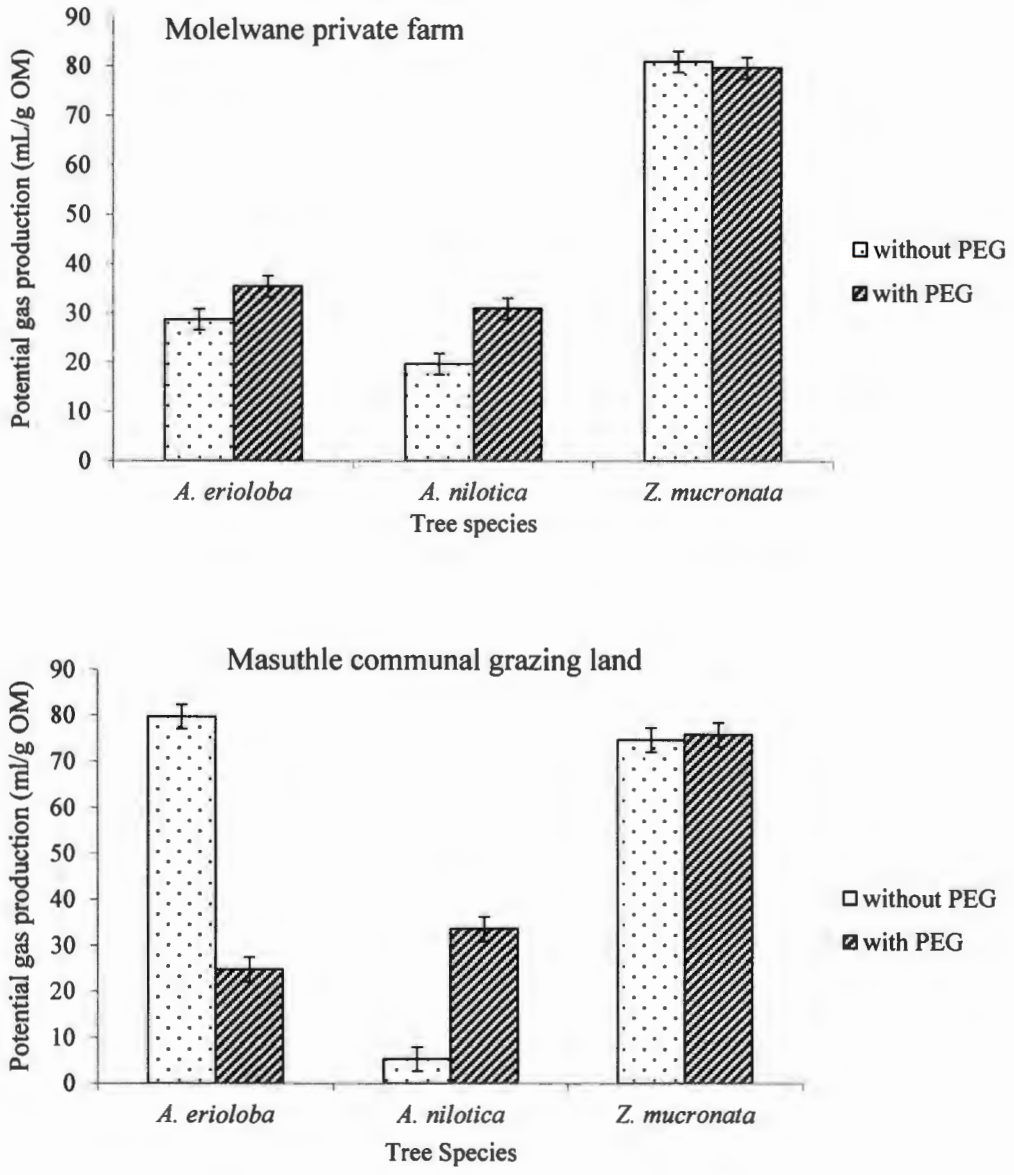


Figure 5.9. The effect of species by PEG interaction on potential gas production (mL/g OM) in leaves harvested from Molelwane private farm and Masuthle communal grazing land

For leaves not treated with PEG, *A. erioloba* and *Z. mucronata* had the higher Pgas produced than the leaves of *A. nilotica*, while for leaves treated with PEG, *Z. mucronata* had the highest Pgas produced followed by the *A. nilotica* and the least was from the leaves of *A. erioloba* (Figure 5.9).

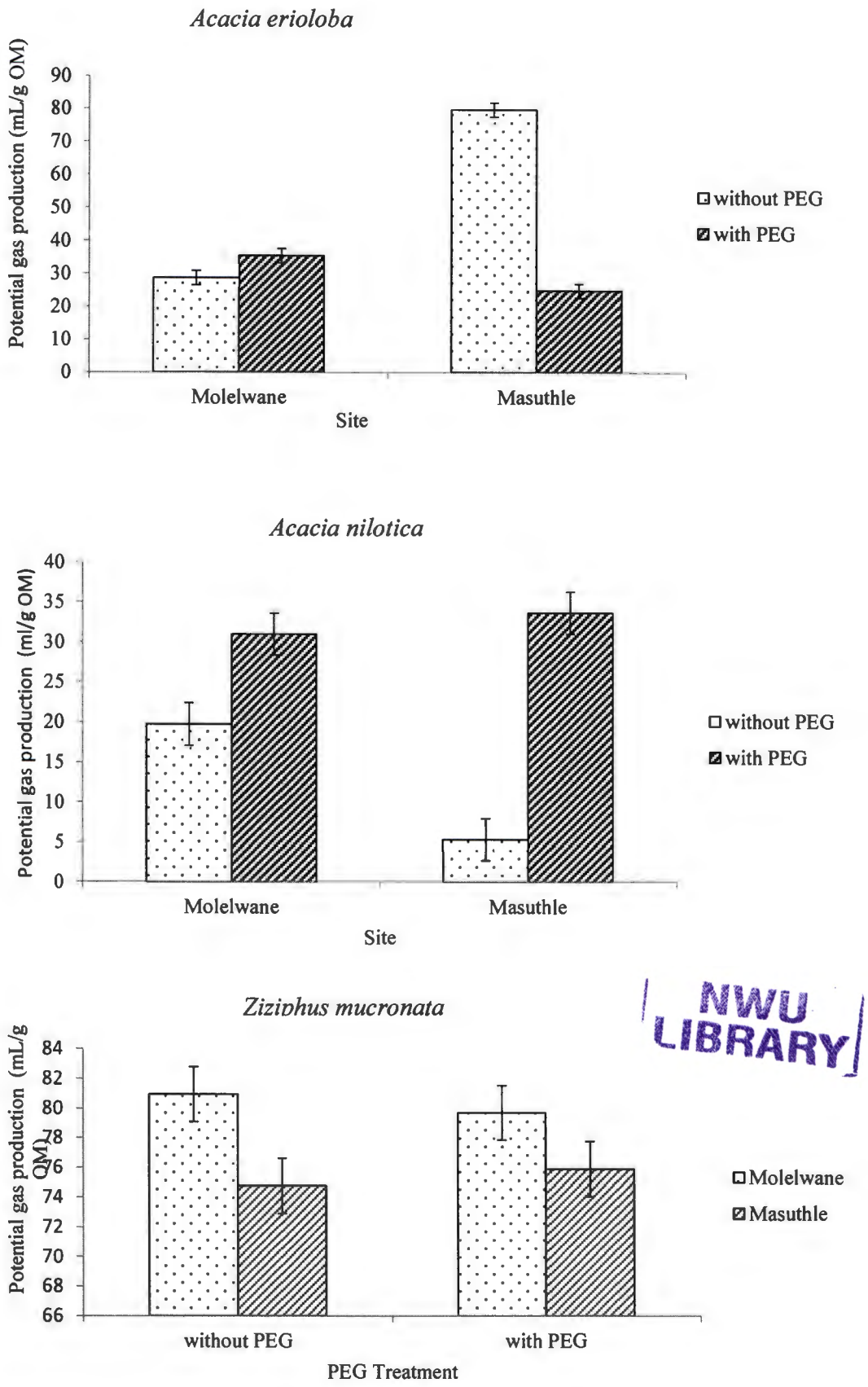


Figure 5.10. The effect of site by PEG interaction from the potential gas production (mL/g OM) in *A. erioloba*, *A. nilotica* and *Z. mucronata* leaves

In Molelwane, *A. erioloba* leaves had the higher Pgas value (35.45 mL/g OM) when treated with PEG than without PEG treatment (28.7 mL/g OM). However, in Masuthle, the leaves of *A. erioloba* had the least Pgas produced (24.8 mL/g OM) when treated with PEG than those without PEG treatment (76.7 mL/g OM). PEG treatment in Molelwane increased the Pgas produced in the leaves of *A. erioloba* while in Masuthle PEG treatment reduced the Pgas produced. In both sites, the leaves of *A. nilotica* had the same Pgas produced when treated with PEG. However, leaves without PEG treatment had a higher Pgas produced in Molelwane (19.7 mL/g OM) than those from Masuthle (5.31 mL/g OM). In Molelwane, the leaves of *A. nilotica* treated with PEG had a higher Pgas (30.96 mL/g OM) than those without PEG treatment (19.7 mL/g OM), and this was also true for the leaves of *A. nilotica* harvested from Masuthle. In both sites, the leaves of *Z. mucronata* had the same (P >0.05) Pgas produced. Leaves of *Z. mucronata* without PEG treatment harvested in Molelwane had a higher Pgas produced (80.93 mL/g OM) than those from Masuthle (74.76 mL/g OM), and this was also true for the leaves of *Z. mucronata* treated with PEG (Figure 5.10).

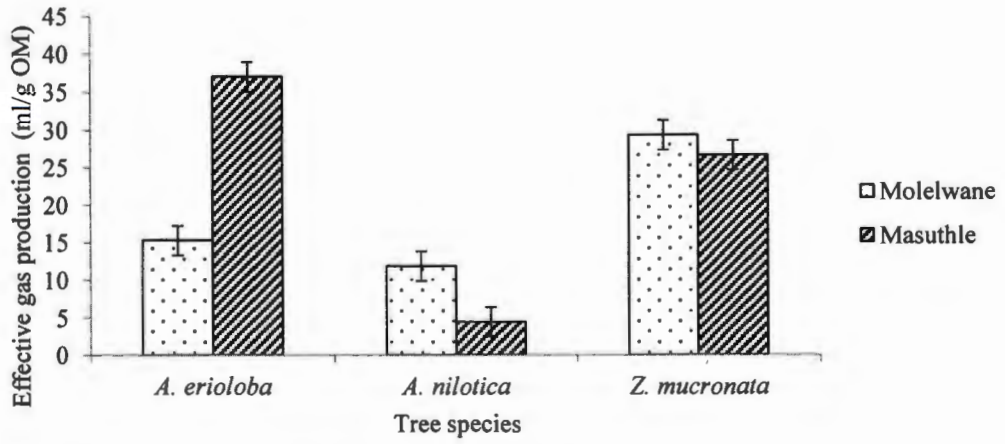


Figure 5.11. The effect of species by site interaction on effective gas (production (mL/g OM) without PEG treatment

Figure 5.11 shows that the untreated *Z. mucronata* leaves had a higher effective gas (Egas) production compared to *A. erioloba* and *A. nilotica* leaves, which did not differ. Leaves of *A. erioloba* harvested from Molelwane had the least Egas without PEG treatment (15.34 mL/g OM) than those harvested from Masuthle (37.08 mL/g OM). The leaves of *A. nilotica* and *Z. mucronata* harvested from Molelwane had the higher Egas produced without the PEG treatment than those harvested from Masuthle.

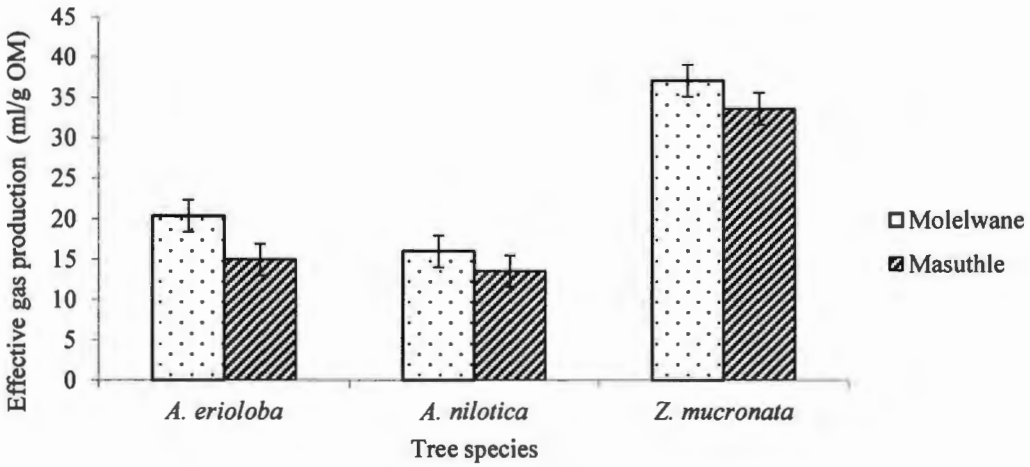


Figure 5.12. The effect of species by site interaction on effective gas production (mL/g OM) from PEG-treated leaves

In Molelwane, leaves of *Z. mucronata* had the highest effective gas (30.08 mL/g OM) produced when treated with PEG, followed by those of *A. erioloba* (20.37 mL/g OM) and *A. nilotica* (16.0 mL/g OM). In Masuthle, the leaves of *Z. mucronata* had the highest Egas values (33.6 mL/g OM) when treated with PEG than those of *A. erioloba* (14.96 mL/g OM) and *A. nilotica* (13.55 mL/g OM), which were statistically the same. Leaves of *A. erioloba* from Molelwane had the higher Egas produced when treated with PEG than those of Masuthle. Apparently, there was no significant (P >0.05) difference between the leaves of *A. nilotica* harvested from both sites. The Egas produced from the

leaves of *Z. mucronata* were the same (P >0.05) for both sites (Figure 5.12). Table 5.4 indicates the levels of significance on the in vitro ruminal organic matter degradability and partitioning factors.

Table 5.4. Statistical significance of the effects of main factors and their interaction on *in vitro* ruminal organic matter degradability (iOMD) and partitioning factors in leaf substrate from *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* tree species harvested in Molelwane and Masuthle

Factors	[1]iOMD	[2]PF
Site	*	NS
Species	***	*
PEG	NS	**
Site × Species	NS	NS
Site × PEG	NS	NS
Species × PEG	NS	NS
Site × Species × PEG	NS	NS

[1]iOMD: *in vitro* organic matter degradability; [2]PF: Partitioning factors

NS: P >0.5; * P <0.05; ** P <0.01; ***P <0.001

The effect of PEG on the 96 h organic matter degradability (g/kg OM) and the partition factors (mL/g OM gas produced) is presented in Table 5.5 below. The *in vitro* organic matter degradability (iOMD) of all the three tree species ranged from 246.2 – 843.8 g/kg OM. Degradability was higher for leaves harvested from Masuthle (552.70 g/kg OM) when compared to those harvested from Molelwane (462.98 g/kg OM). Leaves

harvested from *Z. mucronata* had the highest iOMD content (836.18 g/kg OM), followed by those of *A. erioloba* (394.87 g/kg OM) and *A. nilotica* (292.46 g/kg OM).

Table 5.5. The effect of polyethylene glycol (PEG) on 96 h organic matter degradability (g/kg OM) and partition factors (mL/g OM gas produced) in leaves from *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* tree species harvested from two different sites (Molelwane and Masuthle)

		Molelwane			Masuthle			
[1]Parameters	[2]PEG	[3]AE	[4]AN	[5]ZM	AE	AN	ZM	SE
iOMD	-PEG	246.2^{a}	260.9^{a}	834.7^{b}	436.2^{a}	261.6^{a}	838.2^{b}	77.53
	+PEG	346.8^{a}	261.3^{a}	828.1^{b}	550.4^{ab}	386.1^{a}	843.8^{b}	
PF	-PEG	42.0^{ab}	29.5^{ab}	12.3^{a}	27.7^{ab}	64.1^{b}	13.2^{a}	9.77
	+PEG	11.42^{a}	10.5^{a}	10.03^{a}	14.08^{a}	24.08^{a}	1.52^{a}	

[1]Parameters: iOMD = *In vitro* ruminal organic matter degradability; PF = Partitioning factors

[2]Polyethylene glycol: -PEG = Inoculation without Polyethylene glycol; +PEG = Inoculation with Polyethylene glycol

[3]AE = *Acacia erioloba*; [4]AN = *Acacia nilotica*; [5]ZM = *Ziziphus mucronata*

[a,b] In a row, different superscripts denote significant differences (P <0.05) between tree species

[AB] Uppercase superscripts compare sites for each tree species

The partitioning factors (PF) of all the three tree species ranged from 10.03 – 64.1 mL/g OM. The addition of PEG reduced the PFs in all the three tree species. Leaves of *A. nilotica* had the highest PF content (32.141 mL/mg OM), followed by the *A. erioloba* leaves (23.813 mL/g OM) and the least PF was from the *Z. mucronata* leaves (11.776 mL/g OM).

5.3.2 Relationships between soluble phenolics and condensed tannins

The Pearson's correlation coefficients for the relationship between the PEG effects, SPh and CT are shown in Table 5.6. In Molelwane, there was no significant (P >0.05) correlation between the soluble phenolics and the PEG effect at 24, 36 and 48 hours. However, the condensed tannins had a significant (P <0.05) positive correlation with the PEG effect at 24 and 48 hours, except for the 36 h, which was not significant.

Table 5.6. Pearson's correlation coefficient matrix for linear relationships between PEG effect, SPh and CT of leaves from both sites

Site		PEG effect 24 h	PEG effect 36 h	PEG effect 48 h
Molelwane	[1]SPh	0.390^{NS}	0.343^{NS}	0.002^{NS}
	[2]CT	0.501^{*}	0.215^{NS}	0.751^{***}
Masuthle	SPh	0.388^{NS}	0.113^{NS}	0.052^{NS}
	CT	0.588^{*}	0.630^{**}	0.501^{NS}

[1]SPh: soluble phenolics; [2]CT: condensed tannins

NS: P >0.5; * P <0.05; ** P <0.01; ***P <0.001

In Masuthle, the soluble phenolics also had no significant (P <0.05) correlation with the PEG effect at 24, 36 and 48 hours. However, the condensed tannins had a relatively significant correlation with the PEG effect at 24 and 36 hours, except at 48 h, which was not significant.

5.4 Discussion

The gas production system is currently an outstanding method one can use to measure ruminal gas production in feed stuff and their relationship with the *in vitro* biological activities of tannins because it is automatic and widely used for evaluation. The use of

PEG as a tannin binding compound is a very efficient strategy to mitigate leaf materials that have increased levels of tannins (Waghorn, 2008), especially the leaves of *A. nilotica* which were shown to have a relatively high CT content and high PEG effects particularly those growing in Masuthle. Although, the cost of PEG would limit its use by communal farmers in many small-stock holder sectors, its use can however rather be advantageous when animals are fed with tannin-rich plants. The two sites differed in gas production of the tree leaves. The use of PEG to inactivate CT effects is very useful but can sometimes give confusing results (Waghorn, 2008). Leaves of *A. nilotica* had high amounts of phenolics and they also had high PEG effects, especially those growing in Masuthle. For all tree species and at different time interval, leaves harvested from Masuthle had a higher PEG effect than the leaves harvested from Molelwane, and this may be because the leaves from Masuthle had a high phenolic content than those from Molelwane. The addition of PEG could literally increase gas production. In turn, the increased gas production could also literally mean increased fermentation. According to Makkar *et al.* (1999), a moderate PEG inclusion in a diet resulted in increased DM and N fermentation.

Leaves with higher levels of secondary compounds are likely to decrease the voluntary feed intake and palatability of feed, which suggests that leaves harvested from Masuthle would have had a low voluntary intake than those from Molelwane. According to Mlambo *et al.* (2008), high PF values mean high fermentation efficiency. Table 5.5 shows that with PEG inclusion, leaves had an increased iOMD for all the three tree species, which means that they can be used to predict the microbial protein synthesis in the rumen. On the other hand, a PEG inclusion reduced the fermentation efficiency, and these results were in agreement with the findings of Baba *et al.* (2002) and Mlambo *et al.* (2009), and this may be because PEG decreases the efficiency of microbial protein

synthesis (Makkar *et al.*, 1999). Even though that was the case, leaves from Masuthle had high PFs values than those from Molelwane, which means that the fermentation efficiency of leaves from Masuthle was higher, for example leaves of *A. nilotica* had higher PFs than those from Molelwane. In both sites, the soluble phenolics had no relationship with the PEG effect, and this is because PEG only binds to tannins and not all phenolics. The condensed tannin content in leaves harvested from Masuthle was higher than those from Molelwane and this could explain the higher PEG effect observed in Masuthle leaf substrates. However, at 48 h, the relationship between CT and PEG effect was not significant mainly because PEG effect tends to decline with incubation time. The use of PEG as a tannin-binding compound is an efficient strategy to ameliorate the negative effects of tannins (Waghorn, 2008), particularly for *A. nilotica* in Masuthle, whose leaves were shown to have high CT content. Although the cost of PEG would limit its use by communal farmers in many small-stock sectors, its use can be advantageous when animals are fed on tannin-rich plants. The unfortunate part is that PEG does not bind non-tannin phenolics, which means that these compounds may still interfere with the fermentation of substrates (Mlambo *et al.*, 2009).

5.5 Conclusions

Differences in growth environment did not cause any notable variation in the gas production parameters except for fraction *c*, but the three tree species varied in terms of the tannin content, which could be due to the different levels of foraging pressure across the two sites. In both sites, the soluble phenolics had no relationship with the PEG effect in leaves of all the three tree species. The condensed tannin concentration positively correlated with the PEG effect at various time intervals. The inclusion of PEG caused a reduction of the partitioning factors (PF) but increased the gas production and iOMD for all the three tree species. Leaves harvested from Masuthle generally had

a high fermentation efficiency, especially those of *A. nilotica*, which means that they can be used in high-producing ruminants. Leaves of *A. nilotica* were rich in biologically active condensed tannins that would suppress the rumen microbial activities. These leaves should, therefore, be used with caution when supplementing fibrous diets, which are low in protein. Leaves of *Z. mucronata*, which had the least PEG effect but high CP content can be safely used with fibrous basal diets because they would be able to supply the much-needed rumen degradable protein.

5.6 References

Baba, A.S.H., Castro, F.B. & Ørskov, E.R., 2002. Partitioning of energy and degradability of browse plants *in vitro* and the implications of blocking the effects of tannin by the addition of polyethylene glycol. Anim. Feed Sci. Technol. 95, 93 – 104.

Cornou, C., Storm, I.M.L.D., Hindrichsen, I.K., Worgan, H., Bakewell, E., Ritz, D.R.Y., Abecia, L., Tagliapietra, F., Cattani, M., Ritz, C. & Hansen, H.H., 2013. A ring test of a wireless in vitro gas production system. Anim. Prod. Sci. 53, 585 – 592.

Getachew, G., Blummel, M., Makkar, H.P.S. & Becker, K., 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim. Feed Sci. Technol. 72, 261 – 281.

Makkar, H.P.S., Blummel, M. & Becker, K., 1999. Potential and limitations of *in vitro* gas methods for studying the effects of plant defensive compounds on rumen fermentation. In: Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed. Eds Jansman, A.J.M., Huisman, J. & Van der Poel, A.F.B., Wageningen Press, Wageningen. Pp. 173 – 178.

Makkar, H.PS., 2003. Quantification of Tannins in Tree and Shrub Foliage. A Laboratory Manual. Pp. 1 - 99.

Mauricio, R.M., Mould, F.L., Dhanoa, M.S., Owen, E., Channa, K.S. & Theodorou, M.K., 1999. A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. Anim. Feed Sci. Technol. 79, 321 – 330.

Mlambo, V., Mould, F. L., Smith, T., Owen, E., Sikosana, J. L. N. & Mueller-Harvey, I., 2009: *In vitro* biological activity of tannins from *Acacia* and other tree fruits: correlations with colorimetric and gravimetric phenolic assays. S. Afr. J. Anim. Sci. 39, 131 - 143.

Mlambo, V., Mould, F.L., Sikosana, J. L. N., Smith, T. Owen, E. & Mueller-Harvey, I., 2008. Chemical composition and *in vitro* fermentation of tannin-rich tree fruits. Anim. Feed Sci. Technol. 140, 402 - 417.

Ørskov, E.R. & McDonald, J., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. (Cambridge). 92, 499 - 503.

SAS, 2010. SAS Users Guide: Statistics, Version 9.3 SAS Institute, Cary, NC, USA.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production- Progress and challenges. Anim. Feed Sci. Technol. 147, 116 – 139.

6 CALIBRATION AND VALIDATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY FOR PREDICTING CHEMICAL COMPOSITION AND *IN VITRO* RUMINAL FERMENTATION OF BROWSE TREE LEAVES

Abstract

This study was designed to calibrate and validate the near infrared reflectance spectroscopy (NIRS) technique as a potential tool to predict the nutritional value of browse leaves for ruminants. Leaf samples, harvested and processed, were scanned (32 scans per spectra) from 1100 to 2500 nm with spectra being recorded at intervals of 2 nm using a SpectraStar XL. Spectral data were recorded in diffuse reflectance as log (1/*R*). Reference analyses (chemical composition, buffer-soluble N and *in vitro* ruminal DM degradation) were carried out as described in Section 3, 4, and 5 above. Reference values for all samples were imported into the NIRS spectral data file and used to develop calibration equations. Data analyses and calibration were done with a UCal software (Unity Scientific, Australia). Calibration models were validated using an independent data set of browse leaves harvested in the Eastern Cape Province. Total N and ADF content showed good calibration statistics with high R^2 values of 0.988 and 0.991; and standard error of calibration (SEC) of 0.452 and 13.628, respectively. Calibration models explained 71.1% of the variation in condensed tannins concentration but only 42.0% of the variation in soluble phenolics. Calibration models also explained 91.8 % of the variation in DMD24 but the prediction power for the DMD36 was poor (R^2 = 0.221). External validation showed that the calibration models were able to predict the total N content of independent samples with R^2 and SEP values of 0.82 and 18.19, respectively. However, for all the other nutritional parameters, validation statistics were poor with very low R^2 and high SEP values. It was, therefore, concluded that the NIRS models developed in the current study can accurately be used to

determine the content of total N in browse leaves but not phenolic compounds and *in vitro* ruminal DM degradability.

6.1 Introduction

Near infrared reflectance spectroscopy (NIRS) has been used in recent times to accurately predict the organic composition of conventional feeds. Landau *et al.* (2006) elaborates that NIRS is a non-destructive technique, which does not involve the use of chemicals after calibration is completed. The expansion and development of NIRS has provided a legitimate technique that can estimate the chemical constituents of feed material without the need for laboratory analyses. The use of NIRS also eliminates the need for rumen-fistulated animals, at least after the initial calibration. The application of NIRS is mostly limited to conventional feedstuffs and, certainly, very few studies have been carried out with phenolic-rich tree leaves. This study represents the first attempt to calibrate and validate the NIRS technique using phenolic-rich browse leaves harvested under the semi-arid conditions of the North-West Province. Barton & Windham (1988) suggested that NIRS can be used as a standard technology for the determination of residual moisture, CP, NDF, ADF, crude fat, phenolics and *in vitro* ruminal digestibility. However, for NIRS models to be accurate, sound analytical procedures must be used since these generate the reference values that are used for calibrations. It is, therefore, important to establish reference value data sets that are reliable and accurate for calibration.

The burden to carry out *in vitro* ruminal fermentation studies, which sometimes concern the public, can be simplified by the use of NIRS. Landau *et al.* (2006) amongst other scholars, highlighted that the use of this technique has gained massive recognition from feed industries because its level of accuracy is highly satisfactory and it is a versatile

system for any laboratory involved in animal nutrition research. Many NIRS users have found that the traditional and conventional analyses are more laborious than the NIRS technique in providing an assessment of the calibration model (Foley *et al.*, 1998). This study uses NIRS as a technique to estimate the nutritional composition of browse leaves of *Acacia erioloba*, *Acacia nilotica* and *Ziziphus mucronata* that are growing in the villages of the North-West province (Molelwane and Masutlhe) because no such research has ever been conducted using these trees growing in these areas. Therefore, the objective of the study was to calibrate and validate the near infrared reflectance spectroscopy (NIRS) technique for use in predicting chemical composition and the *in vitro* ruminal fermentation of leaves from *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* tree species harvested from two different growth environments. The hypothesis tested was that the NIRS technique provides spectral variables with nonzero coefficients, which can accurately predict the nutritional value of browse leaves.

6.2 Materials and methods

Leaf samples, harvested and processed as previously described, from *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* were scanned to generate spectral data. Milled leaf samples (0.5 - 1.0 g) were exposed to an electro-magnetic scan over a spectral wavelength range of 1100 to 2500 nm (near infrared) with spectra being recorded at intervals of 2 nm using a SpectraStar XL (Unity Scientific, Australia). Energy in this spectral range is directed onto the leaf sample and the reflected energy (R) is then measured by the instrument. The diffuse reflection carries information which identifies chemical bonds within the sample, such as the -CH, -OH, -NH and -SH bonds. The absorbance associated with chemical bonds in the sample, forms the basis of all organic material and enables the identification of sugars, structural fibres, proteins, lipids and some of their component fractions. Reflectance was converted to absorbance (A) using

the following formula: $A = \log(\frac{1}{R})$ and the spectra were transformed to provide information about the chemical composition of the sample (Baker & Barnes 1990). The NIRS instrument was then calibrated by relating the spectra to chemical components and the *in vitro* ruminal fermentation parameters of the tree leaves. Reference values for all samples were then imported into the NIRS spectral data file and used to develop calibration equations. Data analysis and calibration was done with UCal software. The models from NIRS were then established for DM, OM, N, NDF, ADF, phenolics, and the *in vitro* ruminal fermentation parameters of the leaves from *Acacia erioloba*, *A. nilotica* and *Z. mucronata* tree species.

6.2.1 Calibration and validation

Spectral data were transformed into an Indico pro-format and exported into the UCal software for multivariate data analysis. Principal component analysis (PCA) was performed before partial least square (PLS) regression models were developed. Principal component analysis was used to derive the first principal components from the spectral data, to examine the possible grouping of samples and to detect the possible spectral outliers before using the data set to develop the PLS regression models. None of the mathematical treatments or spectral transformations were applied when PCA was performed. The optimum number of terms in the PLS calibration models were determined by cross-validation and as defined by the prediction residual error sum of squares function to avoid the over fitting of the models (Martens & Naes, 1989). The second derivative was used as a mathematical treatment to correct for the baseline effects and to separate the overlapping peaks when calibration models are developed (Naes *et al.*, 2002). Second derivative was achieved by using Gap-segment transformation and smoothing (5 Gap size and 7 Segment size points). After the second

derivative, the standard normal variation (SNV) transformation was applied. The SNV transformation serves to remove the scatter effects from spectral data and is recommended by several authors when forage samples are being analysed by NIR spectroscopy (Barnes *et al.*, 1989).

Statistics calculated for the calibration models included the coefficient of determination in calibration (R^2) and the standard error of calibration (SEC). The calibration precision was evaluated according to the multiple coefficient of determination, which denotes the proportion of variability in the reference data accounted for by the NIRS regression equation. The SEC was defined as the difference between the estimated and reference values. The calibration accuracy was evaluated by external validation where the initial calibration models were validated using an independent data set of browses leaves of *Acacia karoo*, *Schotia afra*, *Azima tetracantha*, *Cussonia spicata* and *Sideroxylon irreme* harvested at two different canopy heights in the Eastern Cape province of South Africa. This generated additional NIRS performance values for each nutritional component, which included the coefficient of determination and standard error of prediction (SEP). The SEP is the average root mean square difference between predicted and reference values when the NIRS model is applied to an independent data set.

6.2.2 Statistical analysis

The linear regression coefficients of reference versus the predicted values of different nutritional parameters were obtained using Microsoft Excel. The data and spectra used for calibration and prediction were developed using a UCal NIR Calibration Software (Unity Scientific, Australia).

6.3 Results

The results of the calibration of NIRS for chemical composition of leaves from *A. erioloba*, *A. nilotica* and *Z. mucronata* are shown in Table 6.1. Laboratory analyses were used to determine the sample composition in terms of OM, Total N, NDF, ADF, SPh and CT contents.

Table 6.1. Statistics for NIRS calibration results for chemical constituents of browse leaves

	Chemical constituents (g/kg DM)[1]					
[2]Parameters	OM	N	NDF	ADF	SPh	CT
Min	815.429	16.782	195.014	156.334	2.608	0.016
Mean	877.392	23.750	511.886	365.156	36.717	0.280
Max	934.008	33.568	808.574	570.244	82.987	0.817
SD	27.009	4.198	127.697	143.181	17.752	0.213
SEC	17.239	0.452	85.888	13.628	13.520	0.114
R^2	0.593	0.988	0.548	0.991	0.420	0.711
SECV	17.911	0.889	90.315	32.202	15.254	0.119
R^2V	0.478	0.953	0.419	0.939	0.114	0.684

[1]Chemical constituents: OM = organic matter; N = Nitrogen; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; SPh = Soluble Phenolics; CT = Condensed tannins

[2]Parameters: Min = Minimum; Max = Maximum; SD = Standard Deviation; SEC = Standard Error of Calibration; R^2 = Coefficient of determination of Calibration; SECV = Standard Error of Cross Validation; R^2 V = Coefficient of determination of Validation

The high R^2 values for N (0.988) and ADF (0.991) in leaves of the aforementioned tree species indicated excellent predictive capability of the NIRS as compared to the prediction of the other chemical components. Calibration models for condensed tannins

had a higher R^2 (0.711) compared to the models for SPh (0.420), where the predictive power was poor. Moderate R^2 values for OM (0.548) and NDF (0.593) were also obtained.

The calibration statistics for the buffer-soluble N and *in vitro* DM and N degradability, expressed on a DM basis, are shown in Table 6.2. Calibration models predicting the BINSN and BSN had similar R^2 values (0.815). Spectral data predicted a 91.8% of the variation in dry matter degradability at 24 hours (DMD24) but the prediction power for DMD36 was found to be poor ($R^2 = 0.221$).

Table 6.2. Statistics of NIRS calibration results for buffer nitrogen solubility (BINSN and BSN) and *in vitro* ruminal dry matter and nitrogen degradability (DMD24, DMD36, ND24, and ND36) of leaves from browse trees

Parameters[1]	BINSN[2]	BSN[3]	DMD24[4]	DMD36[5]	ND24[6]	ND36[7]
Min	673.8	49.0	175.1	162.1	85.5	44.2
Mean	806.4	193.6	295.38	248.8	231.9	179.5
Max	951.0	326.2	490.0	550.1	563.8	353.2
SD	63.73	63.73	100.9	115.8	105.5	96.4
SEC	27.39	27.39	28.9	102.2	79.8	45.7
R^2	0.815	0.815	0.918	0.221	0.428	0.775
SECV	31.73	31.73	32.99	126.5	103.5	57.4
R^2V	0.714	0.714	0.7738	0.018	0.325	0.596

[1]Parameters: Min = Minimum; Max = Maximum; SD = Standard Deviation; SEC = Standard Error of Calibration; R^2 = Coefficient of determination of Calibration; SECV = Standard Error of Cross Validation; R^2 V = Coefficient of determination of Validation

[2]BINSN = Buffer-insoluble nitrogen; [3]BSN = buffer-soluble nitrogen; DMD24[4] = Dry Matter Degradability at 24 h after inoculation; DMD36[5] = Dry Matter Degradability at 36 h after inoculation; ND24[6] = Nitrogen Degradability at 24 h after inoculation; ND36[7] = Nitrogen Degradability at 24 h after inoculation

The NIRS calibration statistics were less than 50% for the N degradability at 24 h (R^2 = 0.428; SEC = 79.8) and more than 70% for the N degradability at 36 h (R^2 = 0.775; SEC = 45.7).The external validation statistics of the NIRS regression equations obtained from a calibration of the spectral data with chemical components (OM, NDF, ADF, N) and *in vitro* ruminal DM degradability (DMD24 and DMD36) are presented in Table 6.3.

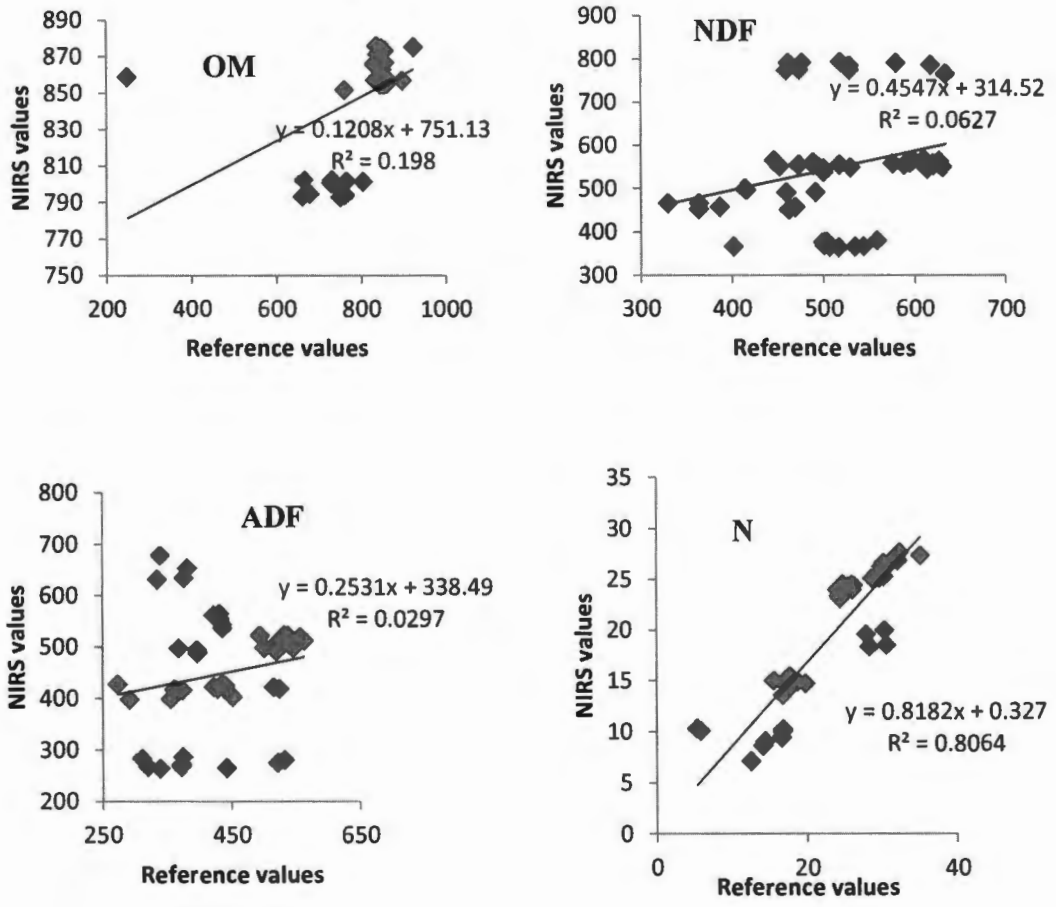


Figure 6.1. Reference versus predicted values for OM, NDF, ADF and N content in an independent data set

The prediction model for total N had the highest R^2 value (0.82) and the lowest SEP (18.19) indicating a good accuracy of the prediction. However, low R^2 values were obtained when models were used to predict the OM (0.2), NDF (0.06), ADF (0.03),

DMD24 (0.1), and DMD36 (0.09) in the independent sample of the browse leaves. Comparisons between the modelled and reference values for the validated independent samples are plotted in Figures 6.1 (chemical components) and 6.2 (dry matter degradability).

Table 6.3. Statistics of validation for calibrated data set using independent spectral data

	Validated Parameters[1]					
Statistical data	OM	NDF	ADF	N	DMD24	DMD36
Samples	50	50	50	50	50	50
Ave. Ref	813.12	509.81	428.03	20.35	371.26	402.29
Ave. NIR	849.38	546.30	446.83	19.51	295.75	281.39
Difference	-36.26	-36.50	-18.80	-17.16	75.50	120.89
Slope	1.64	0.14	0.12	0.10	0.77	1.06
R^2	0.2	0.06	0.03	0.82	0.1	0.09
SEP	96.21	142.69	122.82	18.19	149.39	165.73
SEP(C)	89.12	137.94	121.37	6.03	128.91	113.37
Ave. GD	2.15	3.50	19.99	22.29	1.59	2.48
Ave. ND	0.81	1.47	14.91	17.36	0.56	0.98

[1] Validated Parameters: OM = organic matter; N = Nitrogen; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; DMD24[3] = Dry Matter Degradability at 24 h after inoculation; DMD36[4] = Dry Matter Degradability at 36 h after inoculation

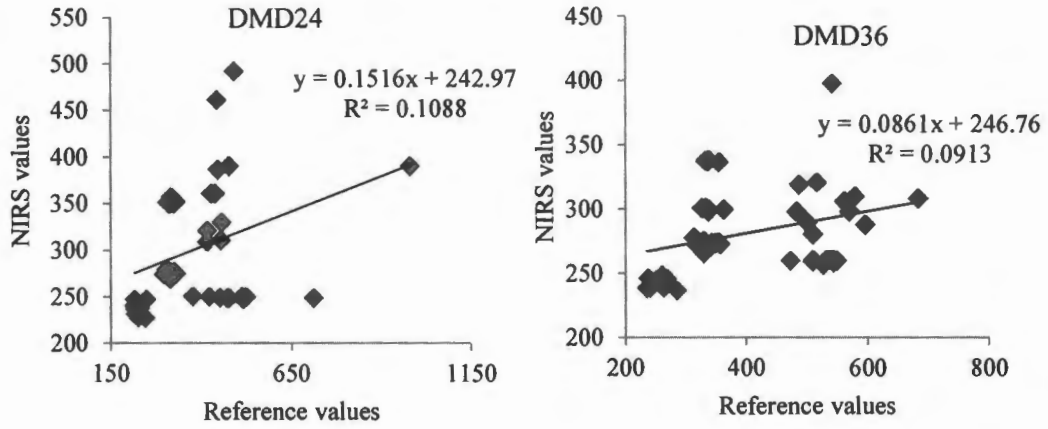


Figure 6.2. Validation of DM degradability at 24 and 36 h from an independent data set

6.4 Discussion

The use of near infrared reflectance spectroscopy as a non-destructive and lower cost analytic technique has attracted more attention from many scholars. This technique offers numerous advantages such as the rapid predictions, the non-use of reagents and the large-scale selection of forages for improved quality (Foley *et al.*, 1998). The utility of NIRS in the assessment of a possible use of tree leaves as potential protein supplements for ruminants was indeed validated by the high R^2 values (> 80%) that accompanied calibration models. According to Foley *et al.* (1998), NIRS cannot change poor analyses (wet chemistry) into good ones and the quality of NIRS-based predictions depends on the size of the spectral data and the quality of the measurements used to generate the statistical model. Corson *et al.* (1999) stated that the application of NIRS can only be as good as the calibration data which is derived from the wet chemistry. Nearly all tested nutritional parameters had R^2 values greater than 50%, with some exceeding 90%, which suggested that NIRS is a potential tool to predict all the chemical components. However, the calibration statistics were poor for soluble phenolics with a low R^2 and SEC (0.420 and 13.520, respectively). The poor

relationship between the soluble phenolic content and the spectral data is surprising given that the relationship with the condensed tannins, a component of soluble phenolics, was quite strong (R^2 = 0.711 and SEC = 0.114). For the DMD24 and DMD36, the prediction using spectral data was not accurate. Corson *et al.* (1999) suggested that the use of NIRS is strongly reliant onto the ability for accurate calibration and sample characteristics that are good enough to provide interpretable spectra. Calibrations alone are not good enough to accurately predict the potential to forage quality, therefore, the need to validate the regression models.

The validation method evaluates the predictive accuracy (Landau *et al.*, 2006). According to Norris (2009), cross-validation demonstrates the potential of NIRS to predict forages. However, the exact accuracy must be estimated with an appropriate data set, which is independent of the calibration set. An independent data set means samples with spectra taken at a different time from the calibration spectra. In this study, leaves harvested from five tree species (*Acacia karoo*, *Schotia afra*, *Azima tetracantha*, *Cussonia spicata* and *Sideroxylon irreme*) growing in the Eastern Cape province of South Africa were used as independent samples. Only OM, NDF, ADF, DMD24 and DMD36 could be validated using this independent sample. Results in Table 6.3 represent some of the chemical components that were validated using the independent data set. The R^2 values for the prediction of OM, NDF, ADF, DMD24 and DMD36 were all less than 20%, reflecting a poor accuracy of prediction. Although NIRS can be a potential predictor of feed chemical constituents, poor wet chemistry from either the calibrated spectra or the independent data set would negate the prediction of the nutritional parameters. Foley *et al.* (1998) concluded that the ability of NIRS to accurately predict chemical components depend on the quality of reference measurements used to generate the statistical model. According to Norris (2009), the

accuracy of predictions may be achieved by using a large number of samples in the final model. One of the limiting factors in this study was that the sample size used for calibration was only 60. The low accuracy of NIRS to predict OM was in line with the conclusion of De Boever *et al.* (1997) that the ability of NIRS to predict OM was not accurate enough. Although NIRS prediction in the independent sample was not convincing for most chemical components and *in vitro* ruminal degradability, the validation was exceptional for the total N content with NIRS model accounting for 82% of the variation in the N content of leaves in the independent sample. This means that the NIRS model for the total N content can be used to accurately predict the total N in browse tree leaves.

6.5 Conclusions

The NIRS calibration models developed in this study had very good statistics (R^2 and SEC), with the exception of the model for soluble phenolics. However, when an external validation was performed using an independent data set, the R^2 values for all parameters, bar total N, declined. This means that NIRS has great potential in predicting the total N in browse leaves although the predictions were limited for the other chemical components (OM, NDF, ADF and DM degradability). Therefore, there is a need to further investigate NIRS as a tool to predict those browse components that had a low accuracy of prediction. A major limitation in this study was the small sample sizes used to generate the NIRS models as well as to validate them. Many scholars have suggested that the use of many samples may generate accurate and relatively reliable results for future predictions. Although this validation might have contained self-loaded errors from the wet chemistry, it gave a preview of the prediction ability of the NIRS technique on browse leaves.

6.6 References

Baker, C.W. & Barnes, R., 1990. The application of near infra-red spectrometry to forage evaluation in the agricultural development and advisory service. In: Feedstuff evaluation. Eds. Wiseman, J., Cole, D.J., London, Butterworths.

Barnes, R.J., Dhanoa, M.S. & Lister, S.J., 1989. Standard normal variate transformation and de-trending of near infrared diffuse reflectance spectra. Appl. Spectrosc. 43, 772 - 777.

Barton, K.E. & Windham,W.R., 1988. Determination of acid detergent fiber and crude protein by near infra-red spectroscopy: collaborative study. J. Assoc. Off. Anal. Chem. 71, 1162 - 1167.

Corson, D.C., Waghorn, G.C., Ulyatt, M.J. & Lee, J., 1999. NIRS: Forage analysis and livestock feeding. Proceedings of the Nes Zealand Grassland Association. 61, 127 – 132.

De Boever, J.L., Cottyn, B.G., De Brabander, D.L., Vanacker, J.M. & Boucque, C.H., 1997. Prediction of the feeding value of maize silages by chemical parameters, *in vitro* digestibility and NIRS. Anim. Feed. Sci. Technol. 66, 211 – 222.

Foley, J.W., Mcllweee, A., Aragones, I.L.L., Woolnough, A.P. & Berding, N., 1998. Ecological applications of near infrared reflectance spectroscopy- a tool for rapid, cost effective prediction of the composition of plant and animal tissues and aspects of animal performance. Oecologia. 116, 293 – 305.

Landau, S., Glasser, T. & Dvash, L., 2006. Monitoring nutrition in small ruminants with the aid of near infrared reflectance spectroscopy (NIRS) technology: A review Small Rumin Res. 61, 1 – 11.

Martens, H. & Naes, T., 1989. Multivariate Calibration. New York: John Wiley and Sons.

Naes, T., Isaksson, T., Fearn, T. & Davies, T., 2002. A User-friendly Guide to Multivariate Calibration and Classification. NIR Publications: Chichester, UK.

Norris, K., 2009. Some considerations about NIR spectroscopy: Closing speech at NIR-2009. J. Near Infrared Spectrosc. 17, 165 – 166.

7 GENERAL DISCUSSION AND CONCLUSIONS

Leaves of *A. erioloba*, *A. nilotica* and *Z. mucronata* were harvested from Molelwane and Masuthle in the North-West province. Molelwane is a private piece of land owned by the North-West University. The grazing system is controlled in this farm with few goats browsing at any particular time. Most of the livestock reared in the farm are given commercial feed, which reduces the time they spend on the natural pastures. On the other hand, Masuthle is an open village with the entire rangeland being exposed to high levels of grazing pressure throughout the year. No controlled grazing is practised in this area. During the lean periods, grasses tend to lose much of their nutrients, particularly protein, and they also deposit more lignin, which reduces their rate of digestion. Apparently and since grasses are a major source of feed for ruminant animals, supplements are needed in times when the quality is poor. Browse leaves remain green and nutritious during the dry seasons, offering animals nutrients as supplements to maintain growth, production and reproduction. The nutritional value of these browse leaves is largely unknown and thus requires some investigation to ensure their possible efficient utilisation. In this study, leaves of *Z. mucronata* had the highest CP content compared to the *Acacia* trees, which means that they can be used in animals whose production levels are high. Under lower production levels, leaves of the *A. erioloba* may be suitable because they had a higher CP content and a low tannin content.

Growth environment influenced the fibre (NDF and ADF) content of the browse leaves but not their mineral matter. Molelwane leaves had a higher mineral content than leaves harvested from Masuthle. Leaves harvested from Masuthle had a high phenolic content than those harvested in Molelwane and this could be attributed to the exposure of browse trees to herbivory. Leaves of *A. nilotica* had a high tannin content, which could be why their solubility index was low because tannins bind to proteins resulting in

insoluble tannin-protein complexes. Tree species caused variation in the *in vitro* ruminal degradability of the leaves but there were no species differences detected in terms of the buffer-soluble N. There was a strong positive relationship between the *in vitro* ruminal DM, the N degradability and the buffer solubility index of N, which means that the buffer-soluble N can be used to predict the *in vitro* ruminal degradability. The study also evaluated the *in vitro* ruminal gas production from leaves and the biological activity of tannins by using a tannin-binding compound polyethylene glycol (PEG). The PEG inclusion could increase both the gas production and *in vitro* OM degradability but reduced the partitioning factors. The highest PEG effect was seen in leaves of the *A. nilotica*, which could be explained by their high CT content. The least PEG effect was seen in leaves of the *Z. mucronata* harvested from Masuthle, suggesting that the CTs in this tree species have a low biological activity against the rumen microbes.

The accuracy of the near infrared reflectance spectroscopy (NIRS) for the purpose of predicting the chemical components and *in vitro* ruminal parameters was also evaluated in Section 6. There was a high R^2 for the calibrated parameters but when an independent set of samples was used for the validation, only N had a high R^2 value (> 80%). The low accuracy of NIRS to predict the other parameters (OM, NDF, ADF, DMD24 and DMD 36) could have been due to the fact that the external validation set of samples was harvested from a different province and came from literally and totally different tree species. In addition, the independent data set was generated by different people at different laboratories. It was then concluded that the NIRS technique can be used as a rapid and non-destructive tool to predict the total N of browse leaves. For NIRS to be accurate, a large sample size of at least 300 samples should be used for

calibrations, and also a large independent sample size must be used for the external validations.

The application of Near Infrared Reflectance Spectroscopy (NIRS) in *in vivo* feeding trials using tree leaves should be further investigated, as this will reveal the true nutritional value of the leaves.

8 APPENDICES

Appendix 1. Recipe for phosphate-borate buffer (Licitra *et al.* 1996)

1. Borate-phosphate buffer, (pH 6.7 - 6.8) including

   a. Monosodium phosphate ($NaH_2OPO_4.H_2O$) 12.20 g/L

   b. Sodium tetraborate ($Na_2B_4O_7.10H_2O$) 8.91 g/L

   c. Tertiary butyl alcohol 100 mL/L

2. Sodium azide 10% solution freshly prepared.

The buffer and the solution were combined by adding 50 mL of borate-phosphate and 1 mL of 10% sodium azide

Appendix 2. Recipe for the *in vitro* ruminal gas production buffer

Reagents

Buffer Solution A:	g/liter
KH_2PO_4	10.0
$MgSO_4.7H_2O$	0.5
NaCl	0.5
$CaCl_2.2H_2O$	0.1
Buffer Solution B:	
Na_2CO_3	15.0
$Na_2S.9H_2O$	1.0

Buffer A and Buffer B were combined using a ratio of 1:5 to adjust pH to obtain a final pH of 6.8

Appendix 3. Analysis of variance tables for chemical components of tree leaves

Factor	Type	Levels	Values
Species	fixed	3	AE; AN; ZM

Analysis of Variance for **DM**, using Adjusted SS for Tests

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	5574.594833	1114.918967	15.21	<.0001
Error	54	3958.775000	73.310648		
Corrected Total	59	9533.369833			

Analysis of Variance for **ASH**, using Adjusted SS for Tests

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	10717.69333	2143.53867	3.96	0.0039
Error	54	29204.89000	540.83130		
Corrected Total	59	39922.58333			

Analysis of Variance for OM, using Adjusted SS for Tests

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	20263.64333	4052.72867	6.85	<.0001
Error	54	31939.16400	591.46600		
Corrected Total	59	52202.80733			

Analysis of Variance for **NDF**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	19228.1802	19228.1802	1.96	0.1668
Species	2	376442.5330	188221.2665	19.23	<.0001
Site*Species	2	67194.5463	33597.2732	3.43	0.0395

Analysis of Variance for **ADF**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SITE	1	10813.8375	10813.8375	9.12	0.0038
SPECIES	2	439796.0970	219898.0485	185.53	<.0001
SITE*SPECIES	2	10013.6010	5006.8005	4.22	0.0197

Analysis of Variance for **N**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	0.0072600	0.0072600	0.00	0.9831
Species	2	644.1010533	322.0505267	20.05	<.0001
Site*Species	2	15.2030800	7.6015400	0.47	0.6255

Analysis of Variance for SPh, using adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	2734.585282	2734.585282	12.83	0.0008
Species	2	512.965481	256.482741	1.20	0.3097
Site*Species	2	339.087152	169.543576	0.80	0.4577

Analysis of Variance for CTs, using adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	0.00450989	0.00450989	0.08	0.7729
Species	2	2.54139527	1.27069763	23.71	<.0001
Site*Species	2	0.35719309	0.17859654	3.33	0.0437

Appendix 4. Analysis of variance tables for buffer-soluble nitrogen and DM and N degradability

Class	Levels	Values
Site	2	MOL MST
Species	3	AE AN ZM

Analysis of Variance for SI, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	39.9250256	39.9250256	1.73	0.1965
Species	2	989.7811333	494.8905667	21.41	<.0001
Site*Species	2	170.7098080	85.3549040	3.69	0.0340

Analysis of Variance for BSN, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	4009.84077	4009.84077	1.73	0.1957
Species	2	98939.47657	49469.73828	21.38	<.0001
Site*Species	2	17031.26452	8515.63226	3.68	0.0343

Analysis of Variance for BINSN, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	4009.84077	4009.84077	1.73	0.1957
Species	2	98939.47657	49469.73828	21.38	<.0001
Site*Species	2	17031.26452	8515.63226	3.68	0.0343

Analysis of Variance for DMD12, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	337.50000	337.50000	0.26	0.6144
Species	2	69270.33250	34635.16625	26.97	<.0001
Site*Species	2	2106.01750	1053.00875	0.82	0.4563

Analysis of Variance for DMD24, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	546.2604	546.2604	0.17	0.6808
Species	2	187490.3808	93745.1904	30.00	<.0001
Site*Species	2	389.4508	194.7254	0.06	0.9398

Analysis of Variance for DMD36, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	66507.4817	66507.4817	155.40	<.0001
Species	2	126252.9733	63126.4867	147.50	<.0001
Site*Species	2	188835.6933	94417.8467	220.62	<.0001

Analysis of Variance for N12, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	479.7204	479.7204	0.05	0.8261
Species	2	120499.1608	60249.5804	6.24	0.0087
Site*Species	2	4474.3258	2237.1629	0.23	0.7954

Analysis of Variance for N24, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	890.13268	890.13268	0.09	0.7648
Species	2	78318.97133	39159.48567	4.07	0.0360
Site*Species	2	1742.49533	871.24767	0.09	0.9139

Analysis of Variance for ND36, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SITE	1	19329.0504	19329.0504	34.30	<.0001
SPECIES	2	129298.9075	64649.4538	114.74	<.0001
SITE*SPECIES	2	2754.6008	1377.3004	2.44	0.1150

Appendix 5. Analysis of variance tables for cumulative gas production, rate of gas production and iOMD of tree leaves with and without PEG

Class	Levels	Values
Site	2	MOL MST
Species	3	AE AN ZM
PEG	2	0 400

Analysis of Variance for **Cumgas12**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	8.559427	8.559427	0.46	0.5033
Species	2	1841.674224	920.837112	48.99	<.0001
PEG	1	983.485040	983.485040	52.32	<.0001
Site*Species	2	147.129665	73.564833	3.91	0.0271
Site*PEG	1	22.356496	22.356496	1.19	0.2813
Species*PEG	2	43.097310	21.548655	1.15	0.3269
Site*Species*PEG	2	58.299527	29.149763	1.55	0.2232

Analysis of Variance for **Cumgas24**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	27.645867	27.645867	0.89	0.3498
Species	2	6247.714170	3123.857085	100.85	<.0001
PEG	1	1642.454685	1642.454685	53.02	<.0001
Site*Species	2	249.379402	124.689701	4.03	0.0246
Site*PEG	1	60.558285	60.558285	1.96	0.1689
Species*PEG	2	61.541530	30.770765	0.99	0.3783
Site*Species*PEG	2	109.037113	54.518557	1.76	0.1837

Analysis of Variance for **Cumgas36**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	90.69465	90.69465	2.37	0.1304
Species	2	10504.20540	5252.10270	137.47	<.0001
PEG	1	1896.61387	1896.61387	49.64	<.0001
Site*Species	2	357.06681	178.53341	4.67	0.0143
Site*PEG	1	117.96206	117.96206	3.09	0.0857
Species*PEG	2	7.98763	3.99381	0.10	0.9010
Site*Species*PEG	2	156.42796	78.21398	2.05	0.1409

Analysis of Variance for **Cumgas48**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	126.63596	126.63596	2.96	0.0922
Species	2	15258.06368	7629.03184	178.30	<.0001
PEG	1	2435.86353	2435.86353	56.93	<.0001
Site*Species	2	534.92106	267.46053	6.25	0.0040
Site*PEG	1	268.41566	268.41566	6.27	0.0159
Species*PEG	2	30.91812	15.45906	0.36	0.6988
Site*Species*PEG	2	121.24373	60.62186	1.42	0.2531

Analysis of Variance for **iOMD**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	140232.360	140232.360	4.67	0.0349
Species	2	3830503.600	1915251.800	63.73	<.0001
PEG	1	55474.695	55474.695	1.85	0.1795
Site*Species	2	109514.699	54757.350	1.82	0.1708
Site*PEG	1	10891.452	10891.452	0.36	0.5495
Species*PEG	2	34282.853	17141.427	0.57	0.5685
Site*Species*PEG	2	11849.639	5924.820	0.20	0.8216

Analysis of Variance for **PF**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	687.629070	687.629070	1.20	0.2781
Species	2	4564.409669	2282.204835	3.98	0.0242
PEG	1	5300.261628	5300.261628	9.25	0.0036
Site*Species	2	2592.704014	1296.352007	2.26	0.1136
Site*PEG	1	7.014083	7.014083	0.01	0.9123
Species*PEG	2	2206.655948	1103.327974	1.92	0.1555
Site*Species*PEG	2	1017.831787	508.915894	0.89	0.4173

Analysis of Variance for ***a***, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	0.06451000	0.06451000	0.09	0.7653
Species	2	41.45973850	20.72986925	29.00	<.0001
PEG	1	3.95512878	3.95512878	5.53	0.0232
Site*Species	2	0.46246783	0.23123392	0.32	0.7253
Site*PEG	1	2.27727744	2.27727744	3.19	0.0812
Species*PEG	2	9.88611153	4.94305577	6.91	0.0024
Site*Species*PEG	2	0.42154811	0.21077406	0.29	0.7461

Analysis of Variance for ***b***, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	51.19672	51.19672	1.57	0.2163
Species	2	29409.11057	14704.55528	451.96	<.0001
PEG	1	1918.63734	1918.63734	58.97	<.0001
Site*Species	2	294.12473	147.06237	4.52	0.0164
Site*PEG	1	421.28435	421.28435	12.95	0.0008
Species*PEG	2	832.55927	416.27964	12.79	<.0001
Site*Species*PEG	2	170.02768	85.01384	2.61	0.0847

Analysis of Variance for *c*, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	0.00109107	0.00109107	9.66	0.0033
Species	2	0.00722531	0.00361266	31.99	<.0001
PEG	1	0.00084972	0.00084972	7.52	0.0088
Site*Species	2	0.00193014	0.00096507	8.55	0.0008
Site*PEG	1	0.00001462	0.00001462	0.13	0.7208
Species*PEG	2	0.00021672	0.00010836	0.96	0.3911
Site*Species*PEG	2	0.00050883	0.00025442	2.25	0.1174

Analysis of Variance for **lag**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	0.32410577	0.32410577	0.32	0.5742
Species	2	26.78049267	13.39024633	13.24	<.0001
PEG	1	2.29525745	2.29525745	2.27	0.1390
Site*Species	2	3.15582813	1.57791406	1.56	0.2214
Site*PEG	1	0.05357638	0.05357638	0.05	0.8190
Species*PEG	2	1.57660981	0.78830491	0.78	0.4648
Site*Species*PEG	2	0.84459904	0.42229952	0.42	0.6612

Analysis of Variance for **Pgas**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	47.62561	47.62561	1.27	0.2653
Species	2	31571.27733	15785.63867	421.97	<.0001
PEG	1	1748.38789	1748.38789	46.74	<.0001
Site*Species	2	312.40706	156.20353	4.18	0.0219
Site*PEG	1	485.48640	485.48640	12.98	0.0008
Species*PEG	2	1023.74299	511.87150	13.68	<.0001
Site*Species*PEG	2	187.20978	93.60489	2.50	0.0935

Analysis of Variance for **Egas**, using Adjusted SS for Tests

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	31.456383	31.456383	2.01	0.1630
Species	2	3643.493810	1821.746905	116.57	<.0001
PEG	1	711.024828	711.024828	45.50	<.0001
Site*Species	2	150.755216	75.377608	4.82	0.0128
Site*PEG	1	45.963651	45.963651	2.94	0.0934
Species*PEG	2	8.713855	4.356928	0.28	0.7580
Site*Species*PEG	2	43.756555	21.878278	1.40	0.2574

Appendix 6. Pearson's correlation coefficients

Pearson's correlation coefficients between solubility index and N degradability in Molelwane

	Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations							
	ND4	**ND6**	**ND12**	**ND24**	**ND36**	**ND48**	**ND72**	**Solubility Index**
ND4	1.00000	0.93231	0.68758	0.65939	0.62223	0.78080	0.48423	0.58091
		<.0001	0.0135	0.0197	0.0307	0.0077	0.1107	0.0476
	12	11	12	12	12	10	12	12
ND6	0.93231	1.00000	0.92995	0.91380	0.70380	0.89347	0.71988	0.58085
	<.0001		<.0001	<.0001	0.0156	0.0012	0.0125	0.0610
	11	11	11	11	11	9	11	11
ND12	0.68758	0.92995	1.00000	0.96306	0.79871	0.79831	0.86317	0.66857
	0.0135	<.0001		<.0001	0.0018	0.0056	0.0003	0.0175
	12	11	12	12	12	10	12	12
ND24	0.65939	0.91380	0.96306	1.00000	0.69775	0.76638	0.77896	0.51742
	0.0197	<.0001	<.0001		0.0116	0.0097	0.0028	0.0849
	12	11	12	12	12	10	12	12
ND36	0.62223	0.70380	0.79871	0.69775	1.00000	0.54721	0.81631	0.91955
	0.0307	0.0156	0.0018	0.0116		0.1016	0.0012	<.0001
	12	11	12	12	12	10	12	12
ND48	0.78080	0.89347	0.79831	0.76638	0.54721	1.00000	0.72590	0.33467
	0.0077	0.0012	0.0056	0.0097	0.1016		0.0175	0.3445
	10	9	10	10	10	10	10	10
ND72	0.48423	0.71988	0.86317	0.77896	0.81631	0.72590	1.00000	0.62473
	0.1107	0.0125	0.0003	0.0028	0.0012	0.0175		0.0299
	12	11	12	12	12	10	12	12
Solubility Index	0.58091	0.58085	0.66857	0.51742	0.91955	0.33467	0.62473	1.00000
	0.0476	0.0610	0.0175	0.0849	<.0001	0.3445	0.0299	
	12	11	12	12	12	10	12	12

Pearson's correlation coefficients between solubility index and N degradability in Masuthle

	Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations							
	ND4	**ND6**	**ND12**	**ND24**	**ND36**	**ND48**	**ND72**	**Solubility Index**
ND4	1.00000	0.93123	0.97498	0.95025	0.97067	0.89868	0.81360	0.50561
		<.0001	<.0001	<.0001	<.0001	<.0001	0.0013	0.0935
	12	11	12	11	11	12	12	12
ND6	0.93123	1.00000	0.96030	0.96249	0.95879	0.87422	0.65508	0.53351
	<.0001		<.0001	<.0001	<.0001	0.0004	0.0287	0.0910
	11	11	11	10	11	11	11	11
ND12	0.97498	0.96030	1.00000	0.98754	0.98719	0.91976	0.73837	0.51109
	<.0001	<.0001		<.0001	<.0001	<.0001	0.0061	0.0895
	12	11	12	11	11	12	12	12
ND24	0.95025	0.96249	0.98754	1.00000	0.99117	0.94941	0.72313	0.53455
	<.0001	<.0001	<.0001		<.0001	<.0001	0.0119	0.0902
	11	10	11	11	10	11	11	11
ND36	0.97067	0.95879	0.98719	0.99117	1.00000	0.94904	0.74926	0.47631
	<.0001	<.0001	<.0001	<.0001		<.0001	0.0079	0.1386
	11	11	11	10	11	11	11	11
ND48	0.89868	0.87422	0.91976	0.94941	0.94904	1.00000	0.81569	0.44676
	<.0001	0.0004	<.0001	<.0001	<.0001		0.0012	0.1454
	12	11	12	11	11	12	12	12
ND72	0.81360	0.65508	0.73837	0.72313	0.74926	0.81569	1.00000	0.38261
	0.0013	0.0287	0.0061	0.0119	0.0079	0.0012		0.2196
	12	11	12	11	11	12	12	12
Solubility Index	0.50561	0.53351	0.51109	0.53455	0.47631	0.44676	0.38261	1.00000
	0.0935	0.0910	0.0895	0.0902	0.1386	0.1454	0.2196	
	12	11	12	11	11	12	12	12

Pearson's correlation coefficients between PEG effect (24, 36 and 48 h), SPh and CT in Molelwane

	PEGeffect24	PEGeffect36	PEGeffect48	SPh	CTs
PEGeffect24	1.00000	0.48910	0.50229	0.38995	0.50129
		0.0545	0.0474	0.1354	0.0479
PEGeffect36	0.48910	1.00000	0.62235	0.34294	0.21519
	0.0545		0.0100	0.1935	0.4235
PEGeffect48	0.50229	0.62235	1.00000	0.00187	0.75149
	0.0474	0.0100		0.9945	0.0008
SPh	0.38995	0.34294	0.00187	1.00000	-0.17151
	0.1354	0.1935	0.9945		0.5253
CTs	0.50129	0.21519	0.75149	-0.17151	1.00000
	0.0479	0.4235	0.0008	0.5253	

Pearson's correlation coefficients between PEG effect (24, 36 and 48 h), SPh and CT in Masuthle

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	PEGeffect24	PEGeffect36	PEGeffect48	SPh	CTs
PEGeffect24	1.00000	0.84601	0.54896	0.38757	0.58832
		<.0001	0.0276	0.1535	0.0165
	16	16	16	15	16
PEGeffect36	0.84601	1.00000	0.84733	0.11318	0.62972
	<.0001		<.0001	0.6880	0.0089
	16	16	16	15	16
PEGeffect48	0.54896	0.84733	1.00000	0.05155	0.50135
	0.0276	<.0001		0.8552	0.0479
	16	16	16	15	16
SPh	0.38757	0.11318	0.05155	1.00000	0.32397
	0.1535	0.6880	0.8552		0.2388
	15	15	15	15	15
CTs	0.58832	0.62972	0.50135	0.32397	1.00000
	0.0165	0.0089	0.0479	0.2388	
	16	16	16	15	16