CHEMICAL COMPOSITION, RUMINAL DEGRADABILITY AND *IN-VITRO* POST RUMINAL DIGESTIBILITY OF *FICUS POLITA AND FICUS BENJAMINA LEAVES*

By

MUFAMADI THAKHANI

STUDENT NO: 11634447

Master of Science in Agriculture (Animal Science)

Department of Animal Science

School of Agriculture

University of Venda

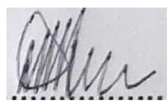
South Africa

Supervisor: Dr F. Fushai	**Signature**	**Date 30th September 2020**
Co-supervisor: Dr M.S. Mikasi	**Signature**	**Date 30th September 2020**

2020

DECLARATION

I, Mufamadi Thakhani, hereby declare that this dissertation submitted to the School of Agriculture for Master of Science in Agriculture (Animal Science) degree at the University of Venda, is my own work and has not been previously submitted for any degree purposes at this or any other university. It is original in design and in execution; all reference material contained herein were duly acknowledged.

Student: Ms T Mufamadi **Signature**  **Date 30th September 2020**

DEDICATION

This dissertation is dedicated to my grandparents, Mr John and Mrs Tshinakaho Ramuluvhana, my father Mufamadi Tshimbiluni Moffat, Mufamadi Ndivhaleni, Nembanzheni Shonisani, Nengwenani Thivhulawi and my lovely uncle, brothers and sisters. I am also dedicating this dissertation to the Lord Almighty.

ACKNOWLEDGEMENTS

This dissertation reflects the work of many people who deserve recognition. I am truly humbled to thank and acknowledge the following:

1. **My heavenly Father, Almighty God**, I thank God for His guidance, love and wisdom during my journey down this path.

2. My **supervisor**, Dr F. Fushai and **co-supervisor**, Dr M.S Mikasi. I am grateful for your patient guidance, encouragement and your support in this research work. Your competent guidance and dedication to help me was irreplaceable in the completion of the study. Your support, perseverance and guidance are sincerely appreciated.

3. **Sponsor**: Special thanks to:

   i. University of Venda Research and Publication Committee Funds (Project No: SARDF/19/ANS/05)

   ii. National Research Foundation for Scarce Skills Scholarship (Grant No: 111757) for offering funds throughout my study.

4. **Family**: I would like to thank my beloved family and Bishop L. Netshivhodza for their support and prayers.

5. **University of Venda**: I am extending my thanks for guidance on chemical analyses offered with kindness by Animal Science Nutrition Laboratory Technician, Mr. M.E. Nyathi.

6. **Colleagues and friends**. My humble gratitude also goes to my teammates Nembanzheni D, Mufamadi K, Mukosi R, Lubisi M.W, Rakau M.V, Mahwasane M.B, Magabo K.N, Ramabusha R and Muhlanga K.T, your constant support is sincerely appreciated.

ABSTRACT

The aim of the study was to evaluate the chemical composition, rumen degradability and *in-vitro* ileal digestibility of dry matter (DM) and nitrogen (N) in *Ficus polita* and *Ficus benjamina* leaves for optimum utilisation as supplementary protein sources for ruminant livestock. Leaves from *Ficus polita* and *Ficus benjamina* were harvested in late summer and in winter from one site within Tshakhuma, Limpopo province, South Africa. Five trees from each species were selected as they randomly occurred along an approximately linear transect of 1 km extending from west to east direction. The leaves were air-dried, milled through a 1 mm screen and analyzed for dry matter (DM), ash, N, neutral detergent fibre (NDF), acid detergent lignin (ADL), acid detergent fibre (ADF) and acid detergent insoluble Nitrogen (ADIN). The DM and N degradability were estimated using approximately 5 g leaf samples which were milled through a 1 mm screen and incubated in duplicate in nylon bags (external dimension: 6 × 12 cm, pore size of 46 µm) inside the rumen of three cannulated Bonsmara steers for 0, 4, 8,16, 24, 48, 72 and 96 hour periods. Parameters to describe the dynamics of ruminal degradability of DM and CP were obtained by fitting the data on the exponential equation $P = a + b\ (1 - e^{-ct})$ using the NEWAY computer program, where "p" is the DM and CP disappearance at time t, (potential degradation), "*a"* is the rapidly degradable fraction, "*b"* is the slowly degradable fraction, "*c*" is the degradability rate of the "*b"* fraction and "t" is the degradation time. Post-ruminal *in vitro* DM and CP digestibility of the rumen undegradable residues were determined by the pepsin-pancreatin (gastric-small intestinal) digestion procedure. Data was subjected to analysis of variance in a 2 (season) x 2 (species) factorial treatment layout. Interaction between the season and species was significant (P<0.01) for Ash, CP, ADF, ADL with the ADF and ADL significantly higher (P<0.01) in winter compared to harvested *F. polita* leaves. For DM, there was a significantly difference for "b" and for the ED at all outflow rates (P<0.01) in both species, and for "a+b" (P<0.05). The insoluble degradable DM fraction '*b*' were higher in *F. polita* than in *F. benjamina* in both dry and wet seasons. The CP disappearance components '*a*' and '*b*' differed significantly ($P < 0.01$) among the two species. *Ficus polita* leaves had significantly higher CP digestibility at 24-hr and 48-hr than *F. benjamina* leaves at 24- and 48-h of rumen incubation in both harvest seasons. It can be concluded that species and season affected the chemical composition, *in-situ* degradability of DM and CP, and *in vitro* post ruminal digestibility of the *Ficus* browse species, with the degradable DM and CP for both species adequate to serve as protein supplements to low-quality ruminant feeds.

Key words: browse trees, digestion, *Ficus*, *in-situ*, Pepsin-pancreatin

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LIST OF ABBREVIATION

a	Soluble Fraction
%	Percentage
^{0}C	Degree Celsius
a + b	Potential Degradability
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
ADIN	Acid Detergent Insoluble Nitrogen
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ARC	Agricultural Research Council
b	Insoluble but Potentially Degradable Fraction
c	Rate of Degradation per hour
Ca	Calcium
CP	Crude Protein
CT	Condensed Tannins
Cu	Copper
DM	Dry Matter
DMI	Dry matter intake
ED	Effective Degradability
Fe	Iron
g	Grams

g/kg	Grams per Kilogram
g/kg DM	Grams per Kilogram Dry Matter
IVDMD	*In vitro* Dry Matter Digestibility
IVCPD	*In vitro* Crude Protein Digestibility
K	Potassium
Mg	Magnesium
mg/kg	Milligram per Kilogram
N	Nitrogen
Na	Sodium
NDF	Neutral Detergent Fibre
P	Phosphorus
RDMD	Rumen Dry Matter Degradability
RCPD	Rumen Crude Protein Degradability
RUP	Rumen Undegraded Protein
RUR	Rumen Undegraded Residues
SEM	Standard Error Mean
Zn	Zinc
Mn	Manganese

CHAPTER ONE

INTRODUCTION

1.1: Background

Nutrition is the major determinant of livestock production in the tropics (Mudzengi *et al*., 2017). Tropical small-holder farmers largely rely on crop residues and low quality foggage to feed ruminant livestock during droughts or the dry seasons (Olubukola *et al.,* 2013). Protein supplements are required to improve the productivity of the livestock. Browsing is also beneficial during periods of feed deficiency, through compensation for deficiencies in protein, vitamins and minerals (Bamikole *et al*., 2004).

There has been a sharp trend in the increase in the cost of feed in recent years, wherein small-scale farmers cannot afford feeds in most African countries (Tshabalala *et al.*, 2013; Herrero *et al*., 2014). Consequently, only few communal farmers can afford to buy supplementary feeds. The potential of browse trees and shrubs as supplementary fodder resources in ruminant nutrition have attracted the attention of researcher's worldwide (Olubukola *et. al.,* 2013). The knowledge of the nutritive value of browse plants that are utilized by ruminants during dry season is therefore indispensable for small-scale farmers.

Ficus Benjamina grows as a large evergreen tree, with up to 10 m wide spreading crowns and drooping shoots, and small twigs (Muhammad *et.al.* 2014). *Ficus polita* grows up to 15 m tall with small leafy twigs, the leaves are spirally arranged together on spurs (a short fat branch) up to 3 cm long (Akesa 2010). *Ficus polita* and *Ficus benjamina* are both native to Tshakhuma village in Vhembe district, Limpopo province, South Africa. *Ficus Polita* (Njidda *et al.,* 2016) and *Ficus benjamina* (Bamikole *et al*., 2004) can be used to provide supplementary protein to ruminants. Despite being an endemic tropical forest species in South Africa, and despite their integration into agro-forestry, *Ficus polita* is underutilized as fodder (Bamikole *et al*., 2004).

1.2: Problem statement

Vhembe District is a drought prone area with a seasonal (summer) rainfall pattern. The area receives an annual rainfall of ± 500 mm that falls predominantly in summer (Rambau *et al.,* 2016). Small-scale farmers in Vhembe District face critical shortages of animal feed, particularly during droughts and dry seasons. Livestock largely depend on poor quality veld grass or hay during these periods. Small scale farmers have limited access to protein supplements for their ruminant livestock and become a constraint during winter and in drought years where there will be poor quality forage, which limits livestock productivity. Consequently, animals lose weight, with low milk yields, poor conception, and increased calf mortalities, all of which culminate into heavy economic loses to smallholder farmers (Ngongoni *et al.,* 2006).

The two *Ficus* species which were investigated in this study are endemic to the area and tolerate arid conditions and provide readily available fodder all year around. The study evaluated the chemical composition, with focus on the protein value of the leaves, including the protein content, its ruminal degradability, and post ruminal digestion, to enable producers to optimize their utilization through application of the most current protein feeding systems for ruminant livestock.

1.3: Justification of the study

The *Ficus benjamina* and *Ficus polita* species which are the subject of this study potentially provide a sustainable option for dry season forage since they are evergreen, but currently not used by farmers as feed for ruminants because of lack of information. These two forages survive in both dry and wet conditions, which allow them to survive throughout the year in Vhembe district. The optimum protein and energy nutrition require quantification of ruminal degradability and post ruminal digestibility of feed components. As a result, analyzing the nutritive value of these *Ficus* species would provide information required by the farmers to improve nutritional efficiency in the production system.

This study presents an opportunity to significantly impact positively on small scale farmers by mitigating the dry season feed deficiency, thereby optimizing the production and quality of animal products to ensure food security for better livelihoods.

1.4: Main objective

The aim of the study was to evaluate the potential for using *Ficus polita* and *Ficus benjamina* leaves as supplementary protein sources for ruminant livestock.

1.5: Specific objectives

The specific objectives were to determine the effects of species (*Ficus polita* versus *Ficus benjamina)* and season (summer versus winter) of harvest on the following:

i. Chemical composition (CP, DM, Ash, ADF, ADL, NDF, ADIN, Ca, P, K and Fe).

ii. Ruminal degradability of dry matter (DM) and crude protein

iii. *In-vitro* post ruminal digestibility of DM and CP.

1.6: Hypotheses

There are no differences between *Ficus polita* and *Ficus benjamina* leaves harvested during summer season (February) and in winter (June-July) in:

i. Chemical composition (CP, DM, Ash, ADF, ADL, NDF, ADIN, Ca, P, K and Fe).

ii. Ruminal degradability kinetics of DM and CP, and

iii. *In-vitro* post ruminal digestibility of DM and CP

CHAPTER TWO

LITERATURE REVIEW

2.1: Introduction

The nutritive value of feeds refers to the amount that can be consumed and the nutrients available to meet the requirements of animals for maintenance, growth and reproduction. The quality of browse is a function of its palatability, intake, digestibility and anti-nutritional factors. Browsable species are highly nutritious and substantially contribute to the diet of livestock in communal areas (Fabian, 2013). Most browse trees have the advantage of maintaining browse production and its nutritive value throughout the dry season when grasses dry up and deteriorate both in quality and quantity (Aganga *et al*., 2005). Grasses produce a first flush of new growth just before the rains (Aganga *et al*., 2005). Tree fodder is readily accepted by livestock and contains high levels of crude protein and minerals, and many show high levels of digestibility (Franzel *et al*., 2014). The deep-root systems enable these browse tree species to produce well into the dry season. However, the anti-nutritive factors can be a problem in some species (Paterson *et al*., 1998). The value of browse trees largely lies in the provision of protein, vitamins and mineral elements that are lacking in grassland pastures during dry season (Bamikole *et al*., 2004). Therefore, the quality and availability of browse trees is very important for livestock production in small-holder farmers during dry seasons and drought years.

In the advent of climate change, beneficial implications on the tropical forage resource (McCollum *et al*., 2017) have been reported. Increasing temperatures and changing rainfall patterns may alter forage production and quality from the tropical rangeland (Oijen *et al*., 2018). For example, as temperature and carbon dioxide (CO_2) levels change, optimal growth ranges for different plants also change; species alter their competition dynamics and the composition of mixed grasslands changes (Fabian 2013). Lack of rainfall and rising temperatures may affect forage digestibility and degradation, with potentially negative impact in livestock production (Fabian 2013). In addition to broadening research to previously excluded species, there is therefore need for continuous evaluation of the nutritive value of a broad range of browse trees in different and in unique environments, to ensure efficient utilisation.

2.2: Nutritive value of browse species

Fodder trees are important sources of high-quality feed for grazing ruminants and as protein supplements to improve the productivity of ruminants fed on low quality feeds (Fabian, 2013). Primary nutritional characterization of forages for ruminants includes the determination of fibre, lignin and the available protein (Givens *et al*., 2000). Good and high quality feeds are the sources of high nutritive value such as CP, NDF, ADF, macro mineral (K, Ca, Mg, S, P) and micro minerals (Fe, Cu, Zn) that promotes live weight gain in livestock. The evaluation of feed chemical components and their degradation characteristics provides important nutritional information, leading to a better understanding of factors that may limit animal performance. In ruminants, the quantity and level of nutrients made available to the animal is dependent on the rumen activity.

2.2.1. Protein and minerals

The nutritive value of forages for livestock feeding depends on the balance between the nutritive components of the plants, the digestibility of such nutrients, the metabolism of absorbed nutrients and the quantity of nutrients ingested by the animal (Lee, 2018). Browsable forages have become increasingly important as protein rich forages to supplement poor quality grass or poor quality roughages for ruminant livestock (Dambe *et al*., 2015). Feed value depends on the content of essential nutrients (protein, fat, carbohydrates, minerals and vitamins) which it contains and proportion which is digested and absorbed.

In addition to the major organic nutrients, a number of macro and micro mineral elements have been shown to be essential for animals (NRC, 2001). Minerals are essential for optimum health for all living species. Requirements differ from one species to the next, but they all need adequate amounts of each mineral for healthy bodily functions. The prominence of each mineral element in the body tissues is closely related to its functional role, as constituents of bones and teeth, minerals provide strength and rigidity to skeletal structures. Many factors affect mineral requirements including nature and level of production, age and chemical form of elements, interrelationship with other nutrients, mineral intake, breed and animal adaptation (McDowell *et al.*, 2012).

Browse trees form a good source of dry-season feed for ruminant animals particularly as protein sources (Bamikole *et al*., 2004). Feed crude protein content of 8% is considered the minimum to meet the rumen microbial ammonium requirement (Goodchild, 1994), beyond which dietary more is required for host animal needs. Njidda *et al.,* (2016) reported high CP content of *Ficus* species (165.30 g/kg DM), enough to supplement poor quality roughages/pastures during the dry season to increase productivity in ruminant livestock. The CP content of the *Ficus* species is within the range of 130.70 – 165.30 g kg DM, which is adequate for the maintenance and growth of grazing ruminants (Njidda *et al*., 2016).

2.2.2. Fibre

The fibre content in these forage species is considered as the primary factor that affects their nutritive value (Njidda *et al*., 2016). Singh and Makkar (1993) attributed variation in efficiency of utilisation among forage species to the fibrous components, such as the structural polysaccharides, which vary in their degradation among forages.

Njidda *et al*., (2016) reported high fibre content of about 536.20 g/kg DM in *Ficus* species forage leaves which will probably result in low effective degradability. It was observed that high NDF and lignification or high ADF/NDF proportion can reduce the digestibility of feed particles by ruminal microbes, as well as inhibit microbial growth and enzyme digestion thus limiting the animal feed intake (McSweeny *et al*., 2001). The NDF content which was reported by Bamikole *et al*., (2004) and Njidda *et al*., (2016) for *Ficus polita,* range from 536.20-654.3 g/kg and *Ficus benjamina* is 677.9 g/kg DM. Feeding forage that may contain high fibre content will reduce animal performance, especially the foggage that farmers rely on during the dry season.

2.3: Forage quality and nutrient requirements of grazing ruminants

A good nutritive value description of forages should include measures of voluntary intake, nutrient or anti-nutrient content, digestibility and metabolism of the nutrients (Mokoboki *et al*., 2019). Most of the browse plants have CP content around 80 g/kg DM in the dry season and ash ranges from 32.5 – 95.8 g/kg DM and can still retain up to 50% moisture in the dry season (Aganga *et al.*, 2005). Browse trees form a good source of dry-season feed for ruminant animals because they are good protein sources (Bamikole *et al*., 2004), however, the low quality of available browse forages may not be sufficient to satisfy their nutritional and energy requirements (Torbit *et al.*, 1985). Browse forages contain crude protein content ranging from 30 g/kg DM to 260 g/kg DM during dry season (Raman, 2003). Most browsable forages have the advantage of maintaining their greenness and nutritive value throughout the dry season when grasses dry up and deteriorate both in quality and quantity (Aganga *et al.*, 2005). Browsable species are highly nutritious and contribute substantially to the diet of livestock in communal areas (Fabian, 2013). The forage quality can be affected by palatability, intake, digestibility and anti-nutritional factors (Lacefield, 2005). Le Houerou (1980) also reported that all browse species are able at all their phenological stages to meet the energy requirements of livestock at maintenance level and often well above. Feeds that contain low crude protein (8%) are deficient because they cannot provide minimum ammonium levels required by animals (Goodchild, 1994). In previous studies, *Ficus polita* contained 952g/kg DM, 108.30g/kg Ash, 165.30g/kg CP, 536.20g/kg NDF, 237.2g/kg ADF and 92.30g/kg ADL (Njidda *et al.*, 2016) while *Ficus benjamina* contained 222.4 g/kg DM, 160.4 g/kg CP and 677.9 g/kg NDF (Bamikole *et al.,* 2004). Njidda *et al.,* (2016) suggested that at 130.70 – 165.30 g kg DM, the CP contents of the *Ficus* species is sufficient to be used to supplement poor quality roughages/pastures during the dry seasons to increase productivity in ruminant livestock.

Feeding forage that may contain high fibre content, especially the forages that farmers rely on during the dry season, reduces animal performance. Overall, the fibre content in these forage species is considered as the primary factor that affects their nutritive value (Njidda *et al*., 2016). According to Singh and Makkar (1993) the statistical variation of forage species is associated with their fibrous components, such as the structural polysaccharides, which vary in their degradation among forages. Njidda *et al*., (2016) reported that the high fiber content of about 536.20 g/kg NDF in these *Ficus* species forage leaves were probably the cause of the low effective degradability. It was observed that high NDF and lignification or high ADF/NDF proportion can reduce the digestibility of feed particles by ruminal microbes, as well as inhibit microbial growth and enzyme activity, which also limit the animal feed intake (McSweeny *et al*., 2001). The fibre content which was reported by Bamikole *et al*., (2004) and Njidda *et al*., (2016) for *Ficus polita,* range from 536.20-654.3 g/kg and *Ficus benjamina* is 677.9 g/kg DM.

2.4. Browse production and voluntary feed intake

Ruminant animals are herbivorous in nature meaning that the largest part of their nutrition comes from grazing on natural pasture and browse forages (Moyo *et al*., 2019). Baumer (1992) stated that, productivity in terms of foliage yield per unit area has been found to be linked with habitat and soil texture. Some browses in favourable humid and sub humid climate situations were reported to produce from 2.3 to 4.69 tons DM forage per hectare per year (Baumer, 1992).

Indigenous browse species, including trees and shrubs, adapted to the existing climatic variability are becoming the major feed resources by supplying protein and energy to maintain livestock production (Anele *et al*., 2009). They reduce seasonal feed resource limitation since they remain green during dry season, produce more biomass and are more nutritious than natural grazing lands (Anele *et al*., 2009; Shenkute *et al*., 2012). In comparison to grasses, browse species are less affected by climatic variability due to their extensive root system and long life-span and they serve as a shelter and fuel as well as maintain and improve the soil fertility (Ahmed *et al*., 2017). *Ficus polita* (Njidda *et al.,* 2016) and *Ficus benjamina* (Bamikole *et al*., 2004) can be used to provide supplementary protein to ruminants. Despite being an endemic tropical forest species in South Africa, and despite their integration into agro-forestry, *Ficus polita* is underutilized as fodder (Bamikole *et al*., 2004). The protein value of *F.* polita and *F.* benjamina browsed in this ecosystem is largely unknown. Non-conventional feeds and forages are feed resources used locally by farmers or have not been traditionally used in commercial or local feeding of livestock (Ahmed *et al*., 2018). These feeds can be available mostly with smallholder farmers and are used for short period of time, especially during the dry season when there is shortage of feeds. Moyo *et al*., (2019) has shown that browse forages are mainly used by smallholder farmers to cope during the dry season. These non-convention forages are used as feed to ruminants, some of them have shown good quality attributes, which can sustain any ruminant livestock. Paudel and Tiwari, (1992) stated that browse species are not susceptible to sudden climatic changes and continue to produce high quality fodder even during drought years. Fodder trees are important sources of high-quality feed for grazing ruminants and as protein supplements to improve the productivity of ruminants fed on low quality feeds (Fabian, 2013). Characterization of forages for ruminants, include the determination of fibre, lignin and the available protein (Givens et al., 2000).

Voluntary feed intake of grazing ruminants is proportional to body weight and metabolic body size (Erfanzadeh *et al*., 2014) and is regulated by both metabolic and physical factors (Aikman *et al*., 2008). If feed intake is limited, production decreases given a greater proportion of consumed metabolisable energy (ME) and nutrients are used for maintenance with limited conversion to animal products Van Do Thi Thanh (2006). If DM of the herbage is lower than 20 %, water volume of the rumen increases and this have a repressive effect on VFI (Meissner *et al*., 1995 and Pasha *et al*., 1994). The higher NDF content is a positive attribute of the browse species since the voluntary DM intake and digestibility are dependent on the cell wall constituent (fibre) and lignin (Bakshi and Wadhwa, 2004).

The NDF, ADF and ADL contents of the *Ficus* species, which ranged from 535.60 – 568.10 g kg^{-1} DM, 215.00 – 246.50 g kg^{-1} DM and 81.0 – 134.10 g kg^{-1} DM respectively, are higher than those reported by Berhane *et al*. (2006), but within the range reported by Abegunde et al. (2008) and Njidda and Ikhimioya (2010) for tropical browse species. Feed intake and feeding behaviour including plant preferences of grazing animals are important determinants of grazing ruminant animal performance (Ahmed *et al*., 2018), which depend on plant species and the environment (Fabian *et al.*, 2013). Plant preference is influenced by the nutrient composition (Khojasteh *et al*., 2013).

2.5: Degradation of browse in the rumen

2.5.1: Ruminal degradation

Rumen degradability is conventionally evaluated *in*-situ by incubation of small feed samples in the rumen in fibre bags of defined dimensions and porosity (Givens *et al.*, 2000). These bags have tiny pores which are small enough to retain the feed sample, but large enough to allow bacteria to enter the bags. It is assumed that the conditions within the bag mimic conditions in the surrounding rumen content. To evaluate degradability, three cannulated Bonsmara steers were used. The rate of ruminal degradability in the browse species was determined by the use of nylon bag technique by Ørskov and McDonald, (1979). Leaves were ground to pass through a 1-mm screen. Approximately 5 g of samples from each treatment was weighed and sealed within 6×12cm nylon bags with a pore size of 46 µm. The bags were attached using plastic bands tied to a flexible vinyl plastic tubes and were then inserted into the rumen of the cannulated Bonsmara steers for incubation for 0, 4, 8,16, 24, 48, 72, and 96 hours. The bags were placed in the rumen in duplicates for each time period simultaneously before the morning feeding. After each time interval, sample bags were withdrawn and washed in running tap water until the water becomes clear, before drying using a forced-air oven at 60^{0}C for 24 hours. The 0 h bags were washed without incubating in the rumen. The washed bags were dried in an oven at 60°C for 24 h and weighed. Dry matter losses were computed as the difference between the determined dry matter content of the pre-incubated samples and the determined dry matter content of the incubated residues. The rumen degradation parameter of DM and CP was calculated using the equations of Ørskov and McDonald (1979):

$P = a + b\,(1 - e^{-ct})$, Where: P = potential degradability after time‘t’; a = water soluble fraction; b = insoluble but degradable fraction after time‘t’; c = rate of degradation of slowly degradable fraction b; t = incubation length i.e. 0, 4, 8, 16, 24, 72, and 96 hours; e = exponential. The effective degradability (ED) was calculated at rumen fractional outflow rates (k) of 0.02, 0.05 and 0.08 per hour by Ørskov & McDonald (1979):

$$\mathrm{ED} = \mathrm{a} + \frac{\mathrm{bc}}{(\mathrm{k} + \mathrm{c})}$$

When feeding ruminant animals, proteins might not be absorbed, but excreted in the faeces as undigestible protein. Therefore, it is important to quantify the protein in these *Ficus* browse species, which is absorbed or utilized by microbes in the rumen by using the nylon bag technique. Kamalak *et al*., (2004) stated that the rate and extent of rumen DM fermentation are very important determinants for the nutrients absorbed by the ruminants. Rumen degradability of protein is an important quantitative measure of the nutritional value of feed protein because it determines the supply of ammonia, peptides and branched-chain fatty acids to ruminal microorganisms, and the passage of undegradable proteins to the intestine (Hvelplund and Weisbjerg, 2000). *In situ* analyses are the most frequently used methods for determination of degradability parameters of DM, organic matter, protein, fibre, minerals and other nutrients of feeds (Harazim *et al*., 2002; Třináctý *et al*., 2003; Čerešňáková *et al*., 2007; Homolka *et al*., 2008; Jančík *et al*., 2009). Mupangwa, (2003) reported variation in the effective degradability of DM in forages, which was correlated with NDF. Haj-Ayed *et al*., (2000) noted that the forage NDF and ADL reduce the effective degradability of DM. Similarly, high fibre content (536.20 k/kg DM) was linked to low effective degradability because fibrous forage is not easily degradable in the rumen (Njidda *et al*., 2016).

Njidda *et al*., (2016) reported that the values obtained for the rapidly degradable fraction ‘a’ of DM were lower than the range of 14%-61% reported by Larbi *et al*. (1998) for browse forages, where in low DM solubility recorded for the browse forage leaves suggests their ability to contribute a substantial amount of bypass nutrients to the hind stomach for the benefit of the animal.

There is limited comparable information on the degradability of *Ficus* browse leaves. Potentially degradable fraction ‘a+b’ of DM in *Ficus* browse leaves was high, above 50% Njidda *et al*., (2016). Effective degradability of DM in the *Ficus* leaves at a rumen out flow rate of 0.12 was highly variable (Njidda *et al*., 2016). Singh and Makkar (1993) attributed the variation to the fibrous components, such as the structural polysaccharides, which vary in their degradation among forages. Mupangwa (2003) observed variations in effective degradability of DM in forages to closely correspond with the proportion of potentially degradable DM and level of NDF. Higher

NDF or ADL reduces effective degradability of browse leaves (Haj-Ayed *et al*. 2000). The higher fibre content recorded in the browse forages leaves may probably be the cause of its low effective degradability (Njidda *et al*., 2016).

Higher values obtained for the outflow rate of degradation (h^{-1}) (c) and potential degradability (a + b) of DM parameters in these *Ficus* browse forages, may indicate a better nutrient availability for rumen micro-organisms in animals in semi-arid areas (Njidda *et al*., 2016). The chemical composition, *in vitro* fermentation and digestibility are largely affected by plant species, plant morphological fraction, environmental factors and stage of growth (Salem, 2005). It was observed that higher ADF/NDF contents can reduce attachment of ruminal microbes to feed particles, inhibiting enzyme activity (McSweeny *et al*., 2001) or intestinal bacterial activity (Salem *et al*., 2004). Njidda *et al*., (2016) reported that the 'a' and 'b' fractions compare favourably with the findings of Abegunde *et al*., (2009) while the 'a+b' was lower (17.00 to 29.33%) compared to the values (43.00 to 58.2%) reported by Abegunde *et al*., (2009) for *Ficus* species. Njidda *et al*., (2016) also stated that the higher values obtained for the 'c' (0.016% to 0.029%) and 'a+b' (71.08% to 82.48%) parameters in the *Ficus* species leaves may indicate an enhanced fermentability and a better nutrient availability for rumen micro-organisms (Elaghandour *et al*., 2016; Getachew *et al*., 2004).

2.5.2: Post ruminal digestibility

The post ruminal digestibility procedure is used to determine the amount of protein utilized in the intestines (Givens *et al*., 2000). Nutrients digestibility in feed samples represents the amount of nutrients in a feed actually accessible to the animal (McDonald *et al*., 2011) therefore, considered as the most essential factor in evaluating the nutritional quality of feed. Nutrients digestibility can be measured using *in vitro* techniques. There is no information regarding the application of *in vitro* methods for protein evaluation in the *Ficus polita* and *Ficus benjamina* leaves.

The three-step procedure (Gargallo *et al*., 2006) closely simulates physiological conditions in the animal. This is achieved by estimating the nutrient digestibility in abomasal and intestinal digestion using pepsin and pancreatin, respectively, from ruminal degradability residues). Because protein may be degraded in the rumen, less digestible dietary protein reaches the abomasum and subsequently the small intestine. The use of estimates of intestinal digestion in combination with estimates of protein degradation in the rumen may provide estimated values of intestinally absorbable dietary protein derived from individual ingredients (Calsamiglia and Stern, 1995).

Beever *et al*. (1974) and Thomson *et al*. (1981) reported that pre-incubating feeds results in reduced amounts of nutrients which will enter post ruminal digestion, in the case referring to *in vitro* stimulation of nutrient digestibility in abomasal and intestinal digestion.

2.6: Summary

Feed supply and quality are some of the major constraints experienced by small-scale farmers in dry seasons. *Ficus* browse species are well adapted to diverse environments and can contribute to significant supplementary protein to livestock diet throughout the year. Hence, the proposed use of these *Ficus* browse species is to ensure regular supply of feed throughout the year. These browse trees provide feed to ruminants because they are productive across different seasons. Previous studies show that the value of CP available in these two browse trees is adequate for maintenance and growth of ruminants. Literature is inconsistent with regard to the efficiency of these browse species in altering the chemical quality, ruminal degradability and these two *Ficus* browsable species have received little attention because their real potentials are still unknown as ruminant feed. Previous studies on the nutritive value of these species are limited to chemical composition and *in-vitro* post ruminal digestibility (Larbi *et al*., 1998; Njidda, 2011). Therefore, further investigation is necessary to determine the chemical composition, ruminal degradability using the nylon bag technique, and *in vitro* digestibility of *Ficus Polita* and *Ficus Benjamina* leaves. This will contribute towards improving livestock productivity for food security and meet the current and future production needs in various environments for better livelihoods.

CHAPTER THREE

COMPARATIVE CHEMICAL COMPOSITION OF *FICUS POLITA* AND *FICUS BENJAMINA* LEAVES

Abstract

Tropical small-scale farmers have limited access to cost effective protein supplements for their ruminant livestock especially during droughts, and during winter, which limits livestock productivity. Browse plants provide significant supplementary protein to grazing ruminants, thus contributing towards improving livestock productivity. The aim of the study was to evaluate the chemical composition of *Ficus polita* and *Ficus benjamina* leaves harvested in two different seasons, for its optimal feeding as protein sources for ruminant livestock. Five trees from each species were randomly selected in the same area as they occurred along a linear transect of 1 km extending from west to east direction within Tshakhuma, Limpopo province, South Africa.The leaves were collected, oven-dried at 60°C, milled to pass through 1 mm sieve and analysed for dry matter (DM), crude protein (CP), Ash, neutral detergent fibre (NDF), acid detergent lignin (ADL), acid detergent insoluble nitrogen (ADIN) and acid detergent fibre (ADF) in the Animal Science Nutrition Laboratory, at the University of Venda. Interaction between the season and species was significant (P<0.01) for Ash, CP, ADF, ADL with the ADF and ADL significantly higher (P<0.01) in winter compared to harvested *F. polita* leaves. The DM and ADIN contents for both species*season interactions were not significant (P >0.05), while the ADF content differed significantly (P < 0.01) and ADL content differed significantly (p<0.05). The ADF 575.8 g/kg and ADL 322.6 g/kg content of *F. polita* were significantly (P<0.01) higher than that of *F. benjamina* 472.9 g/kg and 214.6 g/kg respectively. There was no difference (P > 0.05) in NDF and ADIN for *F. polita* and *F. benjamina* leaves harvested in winter 651.9 g/kg versus 21.8 g/kg and summer 642.7 g/kg versus 22.1 g/kg, respectively. *Ficus polita* recorded higher (P<0.01) CP (206.8 g/kg) in summer compared to winter (150.8 g/kg) and were significantly higher (P<0.01) than the respective summer (143.9 g/kg) and winter (135.5 g/kg) CP content for *Ficus benjamina* leaves. Interaction between the season and species was significant (P<0.01) for Mg, Zn, Mn and Fe content, with the Mg content significantly lower (P>0.05) in winter compared to summer for F. polita, while the difference was not significant for F. benjamina leaves. It can be concluded that, *F. polita* and *F. benjamina* leaves contain high protein levels for use as a protein source for ruminant livestock.

Key words: chemical composition, browse, *Ficus polita*, *Ficus benjamina.*

CHAPTER THREE

3.1: Introduction

Fodder trees provide a potential source of protein for ruminants around the world, which is largely underutilized. Browse forages constitute an important fodder component for ruminants especially in dry seasons, when the available grazing is deficient in both quality and quantity to meet even the maintenance requirements of animals (Aganga and Mosase, 2001). These *Ficus* species can be easily propagated through cuttings, especially *Ficus benjamina* (Ndamitso *et al*., 2010). There is limited information on the chemical composition of *Ficus* browse species in relation to the location and season of harvest, particularly as feed for ruminants. Bamikole *et al*., (2004) reported that the CP content of *Ficus polita* is about 180.2 g/kg and about 160.4 g/kg in *Ficus benjamina,* which suggests adequacy as a protein supplement for ruminants. Browsable tree components should be harvested at peak nutritive value and should be correctly utilized to provide optimal supplementary protein for grazing ruminant livestock.

The aim of the study was to evaluate the chemical composition in *Ficus polita* and *Ficus benjamina* leaves harvested at two different seasons as protein sources for ruminant livestock.

3.2: Materials and methods

3.2.1: Description of the Study Area

Ficus polita and *Ficus benjamina* leaves were harvested in Tshakhuma 23°03'00.0"S 30°18'00.0"E (linear transect of 1 km extending from west to east direction, the area lies east of Louis Trichardt), near Levubu, South Africa. The location receives approximately ± 500 mm of rainfall in summer. The average maximum and minimum temperatures are 31°C and 18°C, respectively (Tadross *et al*., 2005). The region is characterized by deep well drained red clay soils (Soil Classification Working Group, 1991).

3.2.2: Experimental design and sample collection

Leaves from *Ficus polita* and *Ficus benjamina* were in two seasons (summer and winter) in a 2 x 2 factorial experiment in a completely randomized design (CRD). Five tree replicates for each species were randomly selected and marked with cable ties along a linear transect of 1 km extending from west to east direction. The leaves of the selected trees were hand plucked as described by Aster *et al*. (2012) and placed in separate marked brown paper bags. Replicate samples from each tree were plucked to fill 10 brown bags of 41 cm height, 23 cm width and mixed to produce a composite sample. Leaf samples were collected from the same trees in summer (20 February 2019) and during winter season (18 June 2019).

3.2.3: Chemical analysis

The forage samples were analysed at the School of Agriculture, Animal Science Nutrition Laboratory, University of Venda, Thohoyandou, in the Limpopo Province of South Africa. Samples were milled through a 1 mm screen and analysed for dry matter (DM) by drying in an oven at 60^0C for 48 hours (AOAC, 2000; method 976.05). Ash was determined by igniting samples overnight at 550^0C (AOAC, 1990: method 923.03). Nitrogen (N) was analysed using the Kjeldahl method (AOAC, 2000) and it was converted to crude protein as N × 6.25. The NDF, ADF and ADL contents were determined using the method of Van Soest (1991). Acid detergent insoluble nitrogen was determined using the method of Goering and Van Soest., (1970). The leaves were ashed in a CNW Model SXL muffle furnace at a temperature of 600^0C for 24 hours in order to determine individual minerals. The ash materials were then digested in 1M nitric acid. Each digested material was then made to a volume of a 100ml volumetric flask and introduced to the autosampler for analysis of calcium (Ca), phosphorus (P), potassium (K), chlorine (CL) and fluorine (FL). The Varian spectrophotometer instrument (model AA 20) equipped with a graphite tube atomizer (model GTA 110) with a programmable sample dispenser that diluted standards to the required concentration was used. Calibration of Ca and P elements was done using the Merck standards. The three macro elements were then determined by hydride generation technique (AOAC, 2000).

3.2.4: Statistical analysis

Chemical components data was subjected to analysis of variance (ANOVA) for a 2 X 2 factorial experiment using the General Linear Model of MINITAB software version 17 (2014). Differences between means were compared using Tukey's procedure at $P< 0.05$. The statistical model:

$$Y_{ij} = \mu + S_i + B_j + (SB)_{ij} + \varepsilon_{ij},$$

Where:

Y_{ij} = the observation of i^{th} species and j^{th} seasons on DM, Ash, CP, ADF, NDF and condensed tannins,

μ = overall mean,

S_i = effect of the i^{th} species,

B_j = effect of the j^{th} season

$(SBL)_{ij}$ = interaction between i^{th} species and j^{th} season

ε_{ij}, = random error

Means were separated using the Tukey's test at 5% level of significance.

3.4: Results

Chemical composition of *F. polita* and *F. benjamina* leaves harvested in two seasons are presented in Table 3.1. Interaction between the season and species was significant (P<0.01) for Ash, CP, ADF, ADL with the ADF and ADL significantly higher (P<0.01) in winter compared to harvested *F. polita* leaves. The DM and ADIN contents for both species*season interactions were not significant (P >0.05), while Ash, and ADF content differed significantly (P < 0.01) and ADL content differed significantly (p<0.05). The DM and ash content of *F. benjamina* were higher compared to the DM and Ash content of *F. polita* in both seasons. The CP content of *F. polita* was higher than that of *F. benjamina* in both different seasons. The ADIN content of *F. polita* leaves were higher than that of *F. benjamina* leaves.

Table 0.1. Chemical composition (g/kg DM) of *Ficus polita* and *Ficus benjamina* leaves harvested in summer and winter seasons.

Treatments		Chemical Components						
Species	**Season**	**DM g/kg**	**Ash g/kg**	**CP g/kg**	**NDF g/kg**	**ADF g/kg**	**ADL g/kg**	**ADIN g/kg**
F. Polita	1	278.6	123.1[c]	206.8	652.1	539.2[b]	291.8[b]	27.8
	2	298.1	100.2[d]	150.8	679.6	612.3[a]	353.5[a]	26.4
F. Benjamina	1	466.6	181.5[b]	143.9	633.3	481.3[c]	219.5[c]	16.3
	2	445.0	192.8[a]	135.5	624.2	464.6[c]	209.7[c]	17.3
SEM		21.0	0.69	3.04	16.4	7.41	9.6	0.78
Species means								
F. Polita		288.4[b]	111.7[b]	178.8[a]	665.9	575.8[a]	322.6[a]	27.1[a]
F. Benjamina		455.8[a]	187.2[a]	139.7[b]	628.8	472.9[b]	214.6[b]	16.8[b]
SEM		14.8	1.49	2.15	11.6	5.25	6.75	0.55
Season means								
[1]Summer		372.6	152.3[a]	175.4[a]	642.7	510.3[b]	255.6[b]	22.1
[2]Winter		371.0	146.5[b]	143.7[b]	651.9	538.5[a]	281.6[a]	21.8

SEM	14.8	1.49	2.15	11.6	5.25	6.75	0.55
Significance							
Species	**	**	**	*	**	**	**
Season	ns	**	**	ns	**	*	ns
Species*Season	ns	**	**	ns	**	**	ns

**: $P < 0.01$; *: $P < 0.05$; (ns) non-significant: $P > 0.05$.[abcd] Column means with different superscripts differ significantly at $P < 0.05$; SEM: Standard error Mean; DM: dry matter; CP: crude protein; NDF: neutral detergent fibre ADF: Acid detergent fibre; ADL: acid detergent lignin; ADIN: acid detergent insoluble nitrogen; [1]Wet season; [2]Dry season.

The mineral content of *F. polita* and *F. benjamina* leaves are presented in Table 3.2. Season X species interaction was observed for Mg, Zn, Mn and Fe content. The Mg content was significantly lower in winter compared to summer for F. polita leaves. Significant differences were recorded for all minerals ($P < 0.01$) except Na ($P > 0.05$) between the two species. *F. benjamina* leaves had significantly higher ($P < 0.01$) Ca and Fe content than *F. polita* leaves, while *F. polita* leaves showed higher contents for K and P than that of *F. benjamina* leaves in both seasons.

Table 0.2. Macro-mineral (g/kg) and micro-mineral (mg/kg) composition of *F. polita* and *F. benjamina* leaves harvested in summer and winter seasons.

Treatments		**Macro-mineral (g/kg)**					**Micro-mineral (mg/kg)**			
Species	**Season**	**Ca**	**Mg**	**K**	**Na**	**P**	**Zn**	**Cu**	**Mn**	**Fe**
F. Polita	1	21.8^{b}	5.1^{b}	23.7^{a}	0.6	1.7^{a}	21.7^{a}	11.0^{a}	193.3^{a}	163.3^{c}
	2	13.5^{c}	3.8^{c}	21.7^{a}	0.5	1.6^{a}	15.3^{b}	12.0^{a}	134.3^{b}	250.0^{bc}
F. Benjamina	1	44.3^{a}	6.6^{a}	11.3^{b}	0.2	1.0^{b}	10.0^{d}	5.0^{b}	45.0^{c}	535.3^{b}
	2	42.4^{a}	6.4^{a}	9.1^{b}	0.3	0.9^{b}	12.7^{c}	5.0^{b}	49.3^{c}	1348^{a}
SEM		2.61	0.27	0.93	0.2	0.18	0.71	1.12	4.09	109.22
Species means										
F. Polita		17.6^{b}	4.5^{b}	22.7^{a}	0.6	1.6^{a}	18.5^{a}	11.5^{a}	163.8^{a}	206.7^{b}
F. Benjamina		43.3^{a}	6.5^{a}	10.2^{b}	0.3	1.0^{b}	11.3^{b}	5.0^{b}	47.2^{b}	942.0^{a}
SEM		4.04	0.55	1.43	0.18	0.16	2.74	1.07	23.20	331.26
Season means										
[1]Summer		33.02	5.83	17.5	0.42	1.35	15.8	8.5	119.2	349.3
[2]Winter		27.93	5.1	15.4	0.43	1.27	14.0	8.0	91.8	799.0
SEM		5.63	0.64	4.06	0.18	0.17	4.68	3.71	66.31	459.50
Significance										
Species		**	**	**	ns	**	**	**	**	**
Season		**	**	**	ns	ns	**	ns	**	**

Species*season	ns	**	ns	ns	ns	**	ns	**	**

**: $P < 0.01$; *: $P < 0.05$; NS: non-significant: $P > 0.05$; [abcd] Column means with different superscripts differ significantly at $P < 0.05$; Ca: calcium; Mg: magnesium; K: potassium; Na: Sodium; P: phosphorus; Zn: zinc; Cu: Copper; Mn: Manganese; Fe: iron; SEM: Standard error Mean; [1]Summer: wet season; [2]Winter: dry season

3.5: Discussion

The chemical composition and digestibility of forages are influenced by plant species, plant morphological fractions, environmental factors and stage of maturity (Papachristou and Papanaastasis, 1994). In the current study, chemical analysis of *F. polita* and *F. benjamina* leaves harvested in summer and winter showed different values in DM, Ash, CP, NDF, ADL, ADF and ADIN content for both the species and seasons. The respective dry and wet season DM of *F. polita* content was within the previously reported range (295 – 305 g/kg DM), Abegunde *et al.,* (2011), while DM content of *F. benjamina* in both seasons was higher than the one reported by Bamikole, *et al.,* (2004). The ash content was similar to the findings reported by Abegunde *et al*., (2008) who reported ash values ranging from 77.50 - 205.00 g kg^{-1} DM for Ficus species. The lower ash content of *F. polita* leaves in both wet and dry seasons indicated that the plant has a comparatively higher organic matter constituent than *F. benjamina* (Omoregie and Oluyemisi, 2010) due to variation in forage quality among seasons. The content of CP observed in this study (136-207 g/kg DM) was higher than those reported by Bamikole, *et al.,* (2004) and Ndamitso *et al*., (2010). The harvesting season and environmental aspects that include soil type, among other factors, influence the CP content of browse leaves (Ahmed *et al*., 2017). The concentration of CP during both dry and wet seasons in this study are enough to be used as protein source to poor quality matured grasses during dry seasons to improve the productivity of ruminants. The CP content of *F. polita* in wet season was higher than levels observed by Abegunde *et al*., (2011) (165.3 g/kg DM) and Njidda *et al.*, (2016) (19.9 g/100 g). The CP content of these *Ficus* species is above the range of 130.70 – 165.30 g kg^{-1} DM, which was reported to be adequate for maintenance and growth of small ruminants (NRC, 1996) and above 7% level required for microbial activities in the rumen (Norton, 1998). In this study *F. benjamina* CP content in both seasons was lower compared to the CP concentration of *F. polita,* which could be due to variation in forage species, quality among seasons, lower moisture content of the soil and availability of nitrogen, and higher proportion of fibre fraction (Belachew *et al*., 2013).

Fiber contents of these plant species were essential for the optimal functioning of the rumen microbiota. The NDF content in *F. polita* was within the range of 670.0-880.0 g/kg DM that was reported by Abegunde *et al.,* (2011) wherein the quantities are high enough to support well-functioning of those microbiota. The NDF and ADF content in wet season (652.1 g/kg & 539.2 g/kg) were lower than in dry season (679.6 g/kg & 612.3 g/kg) respectively, which was attributed to leaf maturity. However, NDF, ADL and ADF content of *F. benjamina* in both seasons was lower comparing with *F. polita,* which may be related to the different proportions and composition of leaf fractions of the plant. The ADF content of *F. polita* in this study was higher than that reported by Njidda *et al*., (2016), which was (237.2 g/kg). This could be due to environmental variation (Ahmed *et al*., 2017). The ADIN content for *F. polita* was higher than that of *F. benjamina* in both wet and dry season. Dry season ADIN contents in these *Ficus* browse species are partially high than those of wet season because the proportion of nitrogen found as acid detergent insoluble nitrogen (ADIN) increases with the increasing forage maturity (Coblentz *et al*., 1998 and Elizalde *et al.,* 1999). This is because as the plant matures, it deposits more of its tissue as lignin to provide structural support (Oba and Allen, 1999).

Minerals are required for nutrient metabolism and for proper functioning of organs. NRC (1976) recommended 3.0 g/kg as the critical level of Ca in the diet of ruminants. However, the Ca content of these *Ficus* species is high than the recommended by NRC (1976), thus making them to be good source of Ca. *F. benjamina* leaves in both seasons was observed to have higher Ca and Mg content than those of *F. polita.* The K mineral content for *F. polita* leaves was recorded to be higher than *F. benjamina* K content in both seasons, therefore, this browse leaves had high K content when comparing to the recommended critical requirement level for grazing animals by Underwood, (1981). High-producing ruminants or those under stress such as heat stress may require K levels above 10 g/kg (Rambau *et al*, 2016). Excessive K content induces deficiency of other minerals and immune suppression and K levels of 6 – 8 g/kg are considered to be adequate for cattle (Mirzaei, 2012). However, the results of this study indicate that F. polita leaves would not need K supplementation when they are fed to ruminants, while F. benjamina leaves would require K supplementation. In the present study, K levels were high which may cause antagonism to other minerals and multiple mineral imbalance and induce deficiencies may occur (Mirzaei, 2012).

Abdullah *et al*. (2013) recommended the range of 1.2 – 4.8 g/kg P for all classes of ruminants. In the current study, the P content level for *F. polita* is within the recommended range while that for *F. benjamina* leaves was lower than the recommended range. To meet the need of highly productive animals, forage should contain more than 1.5 g/kg of sodium (Na) and Na deficiency is more likely to occur in animals grazing on tropical pasture species and these species generally accumulate less Na than temperate species (Morris, 1980). Zn and Cu concentrations for *F. polita* were higher than *F. benjamina*. Manganese (Mn) concentration in the present study for these browse species exceeded the recommended levels of 20 – 25 mg/kg for cattle and sheep and 40 mg/kg, the critical levels found to be sufficiently higher to meet the requirements of ruminants (NRC 2000 and NRC 2001). The iron (Fe) concentration of *F. benjamina* leaves in dry season that was found in this study is in an agreement with the higher forage Fe value of 650 mg/kg reported by Khan (2003). Le Houerou (1980) also noted that all browse species are able at all their phenological stages to meet the energy requirements of livestock at maintenance level and often well above.

3.6: Conclusion

Season and species affected the chemical composition of the two-browse species. High concentrations of CP were observed in *F. polita* during both dry and wet seasons. The CP content qualified the forages as protein supplements to poor quality matured grasses during dry seasons to improve the productivity of ruminants.

CHAPTER FOUR

RUMEN DEGRADABILITY OF *FICUS POLITA* AND *FICUS BENJAMINA* LEAVES

Abstract

Browse plants provide significant supplementary protein to grazing ruminants, thus contributing towards improving livestock productivity. The objective of this study was to evaluate the ruminal dry matter (DM) and crude protein degradability of *Ficus polita* and *Ficus benjamina* leaves harvested in two different seasons. Five trees from each species were selected as they randomly occurred along an approximately linear transect of 1 km extending from west to east direction Tshakhuma, Limpopo province, South Africa. The leaves were collected, oven-dried, milled to pass through 1 mm sieve and analysed for DM and nitrogen (N) in the Animal Science Nutrition Laboratory, at the University of Venda. Approximately 5 g of samples were placed in nylon bags (46 µm) and incubated in duplicates for 0, 4, 8, 16, 24, 48, 72 and 96 hour periods in the rumen of three cannulated Bonsmara steers. The residues were then analysed for DM and nitrogen. Parameters to describe the dynamics of ruminal degradability of DM and CP were obtained by fitting the data on the exponential equation $P = a + b\ (1 - e^{-ct})$ using NEWAY computer program, where "*a"* is the rapid degradable fraction, "*b"* is the slow degradable fraction and "*c*" is the the fractional rate of degradation of fraction *b*. For DM, there was a significantly difference for "b" and for the ED at all outflow rates (P<0.01) in both species, and for "a+b" (P<0.05). The insoluble degradable DM fraction '*b*' were higher in *F. polita* than in *F. benjamina* in both dry and wet seasons. The potentially degradable DM '*a+b'* value was greater in *F. polita* (58.9% and 56.5%) in wet and dry season respectively, compared to *F. benjamina* with 51.2% in wet season and 51.5% in dry season. Effective degradability increased (P < 0.01) with an increase in the outflow rates. Significant (P<0.01) interaction was observed for all parameters of CP degradation except for the rate constant "c". The ED at outflow rates 0.02, 0.05 and 0.08 for both *Ficus* species were (42.4% and 34.7%), (31.3% and, 24.7%) and (25.4% and 19.7%) respectively. In conclusion, there was significant difference between the two *Ficus* browse species in CP degradability and considered adequate to support rumen microbes to ensure effective rumen function.

Key words: Dry matter, Crude protein, Ruminal degradability, *Ficus*.

CHAPTER FOUR

4.1: Introduction

Rumen degradability is conventionally estimated using the *in-situ* method, by incubation of small feed samples in the rumen in fibre bags (Ørskov *et al*., 1980). Degradability determines the disappearance of feeds incubated in a porous bag in the rumen of the cannulated animal to estimate both the extent and rate of degradation. The rate and extent of degradation in the rumen is an important parameter which determines the supply of dietary nutrients both to the rumen microbes and ultimately, to host animal body tissues (Mohamed and Chaudhry, 2008). The *in-situ* technique has been used to rank feeds according to the rate and extent of degradation of dry matter, organic matter, nitrogen, neutral detergent fibre and acid detergent fibre (Harazim *et al.,* 2002; Třináctý *et al*., 2003; Čerešňáková *et al*., 2007; Homolka *et al.,* 2008; Jančík *et al*., 2009; Rambau *et al.,* 2016).

The two *Ficus* browsable species have so far not been comparatively evaluated in a similar manner for use as protein supplements for ruminants. Very little is known on the ruminal degradability of *Ficus* browse species in relation to its quality when harvested in different seasons. The aim of the study was to determine the ruminal dry matter (DM) and crude protein degradability of *Ficus polita* and *Ficus benjamina* leaves harvested in two different seasons.

4.2: Materials and methods

4.2.1: Leaf sampling, sample preparation and chemical analyses

Leaves from *Ficus polita* and *Ficus benjamina* were sampled and harvested as described in chapter 3. For analysis of chemical components, the leaves were ground to pass through a 1-mm screen and approximately 5 g of samples was weighed and put in nylon bags. The leaves were analysed in the University of Venda experimental farm and Animal Nutrition Lab (22^{0}56’60’’ S, 30^{0}28’60’’ E), Thohoyandou, Limpopo, South Africa.

4.2.2: Management of animals and ethical consideration

Approval was sought from the Ethics Committee of the University of Venda (SARDF/19/ANS/05) before the research trials with animals start. Approximately 3-year-old Bonsmara steers with mean live weight of 420±SD kg which were surgically fitted with rumen cannulae of 10 cm centre diameter (ANKOM-flexible cattle purchased from Bar Diamond Inc.) were used to determine the degradability profiles of DM and CP of *Ficus polita* and *Ficus benjamina* leaves. The animals were kept in feeding pens in a feedlot facility at the University of Venda. They were fed a complete cattle finisher diet (Table 4.1) twice a day, starting 21 days prior to the commencement of the incubation of nylon bags. Clean drinking water was always available in water troughs.

Table 0.1. Chemical composition of the commercial complete cattle finisher diet.

Composition	**g/kg**
Protein (min)	120
Calcium (max)	8
Phosphorus (min)	3
Moisture (max)	120
Fibre (max)	200
Fat (min)	25
Urea (max)	12.5
% Derived from urea	29.9%
	mg/kg
Monensin NA	30
Zinc Bacitracin	50

*Supplied by Driehoek Feeds (Vaalwater, Waterberg, Limpopo, South Africa)

4.2.3: Experimental design and Rumen incubation procedure

Forage samples were obtained as per factorial experiment which was described in section 3.2.4. To evaluate degradability, three cannulated Bonsmara steers were used. Ruminal degradability was determined using the nylon bag technique by Ørskov and McDonald, (1979). Leaves were ground to pass through a 1-mm screen. Approximately 5 g of samples from each treatment was weighed and sealed within 6×12cm nylon bags with a pore size of 46 µm. The bags were attached using plastic bands tied to a flexible vinyl plastic tubes and were then inserted into the rumen of the cannulated Bonsmara steers for incubation for 0, 4, 8,16, 24, 48, 72, and 96 hours. The bags were placed in the rumen in duplicates for each time period simultaneously before the morning feeding. After each time interval, sample bags were withdrawn and washed in running tap water until the water becomes clear, before drying using a forced-air oven at 60^0C for 24 hours. The 0 h bags were washed without incubating in the rumen. The washed bags were dried in an oven at 60°C for 24 h and weighed.

4.2.4: Chemical analyses for CP

The washed bags were dried in a forced air oven at 60°C for 48 h (AOAC, 1990; method 930.15), desiccated for 30 minutes and then weighed to determine DM content. The final residues in all bags were composited by the species treatment, incubation hour and steers and subsequently ground through a 1 mm sieve and analysed in duplicates. The residues were then analysed for Nitrogen (N) content using the Kjeldahl procedure (AOAC, 1990; method 984.13) and N was converted to CP as N x 6.25.

4.2.5: Mathematical calculations

The degradability of dry matter and protein with time for each sample was described using the mathematical model by Ørskov and McDonald (1979).

Equation:

$$\text{Degradability} = \frac{\text{initial mass} - \text{final mass}}{\text{initial mass}} \qquad \text{Equation 1}$$

The dry matter and N data were fitted into the model

$$P = a + b\,(1 - e^{-ct}) \qquad \text{Model 1}$$

Where:

P = fraction of DM/protein degraded at time t of incubation

a = fraction of immediately degradable (soluble) DM/protein

b = fraction of not soluble, but degradable DM/protein

c = the fractional rate of degradation of fraction b

Potential degradability (PD) were estimated as (a + b). Effective degradability (ED) was calculated at rumen fractional outflow rates (k) of 0.02, 0.05 and 0.08 per hour by Ørskov & McDonald (1979):

$$ED = a + \frac{bc}{(k + c)} \quad \text{Model 2}$$

Where:

ED = Effective degradability

a = constant; immediately degradable (soluble) protein

b = constant; slowly degradable

c = fractional rate of degradation of constant *b*

k = estimated rumen outflow rate of 0.02, 0.05 and 0.08

4.2.5: Statistical analysis

All parameters were subjected to analysis of variance (ANOVA) using the General Linear Model of MINITAB software version 17 (2014). Significant difference between the means were compared (at 5% level of significance) using the Tukey's procedure.

The statistical model:

$$Y_{ijk} = \mu + S_i + B_j + (SB)_{ij} + \varepsilon_{ijk} \quad \text{Model 3}$$

Where Y_{ijk} = the observation of i^{th} species, j^{th} season on DM and CP,

μ = overall mean common to all observations;

S_i = effect of the i^{th} species,

B_j = effect of season on i^{th} species,

$(SB)_{ij}$ = interaction between i^{th} species and j^{th} season,

ε_{ij}, = random error

Means were separated using the Tukey's test at 5% level of significance.

4.3: Results

4.3.1: Degradability constants and Effective degradability

In situ DM disappearance (%) of *F. polita* and *F. benjamina* leaves harvested in wet and dry seasons is illustrated in Figure 4.1. Parameters descriptive of the DM and CP degradability kinetics were estimated by fitting the model of Ørskov and McDonald (1979), defining the kinetics of DM and CP degradation and effective degradability at three rumen fractional outflow rates, are presented in Table 4.2. For DM, there was a significantly difference for "b" and for the ED at all outflow rates (P<0.01) in both species, and for "a+b" (P<0.05). The insoluble degradable DM fraction '*b*' were higher in *F. polita* than in *F. benjamina* in both dry and wet seasons. The potentially degradable DM '*a+b'* value was greater in *F. polita* (58.9% and 56.5%) in wet and dry season respectively, compared to *F. benjamina* with 51.2% in wet season and 51.5% in dry season. Effective degradability was significantly higher (P<0.01) for *F. polita* compared to *F. benjamina* in both seasons and all rumen outflow rates. The season effect was observed at 2% rumen fractional outflow rate with the wet season higher than the dry season. There was no significant difference in season*species interaction across all DMD parameters. However, effective degradability (%) decreased when the rate of passage was increased from 2% to 8%.

Significant (P<0.01) interaction was observed for all parameters of CP degradation except for the rate constant "c". Significant (P<0.01) species and seasonal effects were also observed in all crude protein degradability parameters except on the (c) outflow rate of degradation (h^{-1}). The effective digestibility of *F. benjamina* was higher than that of *F. polita* in all rumen outflow rates. The crude protein soluble fraction (a) and potential degradability (*a* + *b*) values for *F. benjamina* were significantly higher (P<0.01) than of *F. polita* in both seasons. The CP soluble fraction was higher in dry season than in the wet season for both species, however, factor interactions were noticed as the margin of increase was significantly higher for *F. benjamina* than *F. polita*. The insoluble but potentially degradable CP fraction (b) was higher for *F. polita* compared to *F. benjamina* in wet seasons. There was significant difference in season*species interaction across all CPD parameters (P,0.01), except for 'c' (P>0.05). However, effective degradability (%) was significantly different (P<0.01) for 5% and 8%, except for 2% (P<0.05).

Table 0.2. Degradability of *F. polita* and *F. benjamina* leaves harvested in summer and winter seasons.

Treatment		Degradability parameters (%)				ED (%) at different outflow rates		
Species	**Season**	***a***	***b***	***c***	***a + b***	***k* =0.02**	***k* = 0.05**	***k* = 0.08**
Dry Matter								
F. Polita	1	6.9	52.1[a]	0.046	58.9	43.1[a]	31.5[a]	25.9[a]
	2	5.2	51.2[a]	0.051	56.5	41.6[b]	30.7[a]	24.9[a]
F. Benjamina	1	5.5	45.7[b]	0.039	51.2	35.6[c]	25.5[b]	20.4[b]
	2	5.9	45.5[b]	0.033	51.5	33.8[d]	23.7[b]	18.9[b]
SEM		1.47	1.19	0.0078	2.22	0.17	0.56	0.53
Species means								
F. Polita		6.0	51.7[a]	0.049	57.7[a]	42.4[a]	31.3[a]	25.4[a]
F. Benjamina		5.7	45.6[b]	0.036	51.3[b]	34.7[b]	24.7[b]	19.7[b]
SEM		1.04	1.43	0.0056	1.57	1.02	1.08	0.35
Season means								
[1]Summer		6.2	48.9	0.043	55.05	39.4	28.7	23.2
[2]Winter		5.6	48.3	0.042	53.93	37.7	27.2	21.9
SEM		1.04	1.43	0.0056	1.57	4.43	3.93	0.35
Significance								
Species		ns	**	ns	*	**	**	**
Season		ns	ns	ns	ns	ns	ns	ns
Species*season		ns	ns	ns	ns	ns	ns	ns
Crude Protein								
F. Polita	1	11.4[d]	80.0[b]	0.049	13.8[d]	10.0[d]	12.9[d]	10.8[d]
	2	10.1[c]	40.0[a]	0.055	15.2[c]	14.1[c]	15.2[c]	12.8[c]
F. Benjamina	1	15.8[b]	70.2[c]	0.071	16.5[b]	16.4[b]	16.2[b]	16.1[b]
	2	75.8[a]	70.7[c]	0.072	76.5[a]	19.3[a]	19.2[a]	19.2[a]
SEM		0.06	0.4	0.009	0.05	0.045	0.05	0.05

Species means							
F. Polita	10.8^{b}	60.0^{a}	0.051	14.5^{b}	12.1^{b}	14.1^{b}	11.8^{b}
F. Benjamina	45.8^{a}	70.5^{b}	0.072	46.5^{a}	17.8^{a}	17.7^{a}	17.6^{a}
SEM	0.3	0.01	0.006	0.02	0.02	0.3	0.3
Season means							
[1]Summer	13.6^{b}	75.1^{b}	0.060	15.2^{b}	13.2^{b}	14.5^{b}	13.5^{a}
[2]Winter	42.9^{a}	55.4^{a}	0.064	45.8^{a}	16.7^{a}	17.2^{a}	16.0^{b}
SEM	0.3	0.01	0.006	0.02	0.02	0.3	0.3
Significance							
Species	**	**	ns	**	**	**	**
Season	**	**	ns	**	**	**	**
Species*season	**	**	ns	**	*	**	**

**: P <0.01; *: P <0.05; (ns) non-significant: P >0.05. ^{abcd} Column means with different superscripts (for Dry Matter and Crude Protein degradability parameters separately) differ significantly at P <0.05. a: soluble fraction, b: insoluble but potentially degradable fraction, a + b: Potential degradability, c: outflow rate of degradation (h^{-1}), ED: effective degradability, K: rumen outflow rate (h^{-1}), %: Percentage and SEM: Standard Error Mean. [1]Wet season; [2]Dry season.

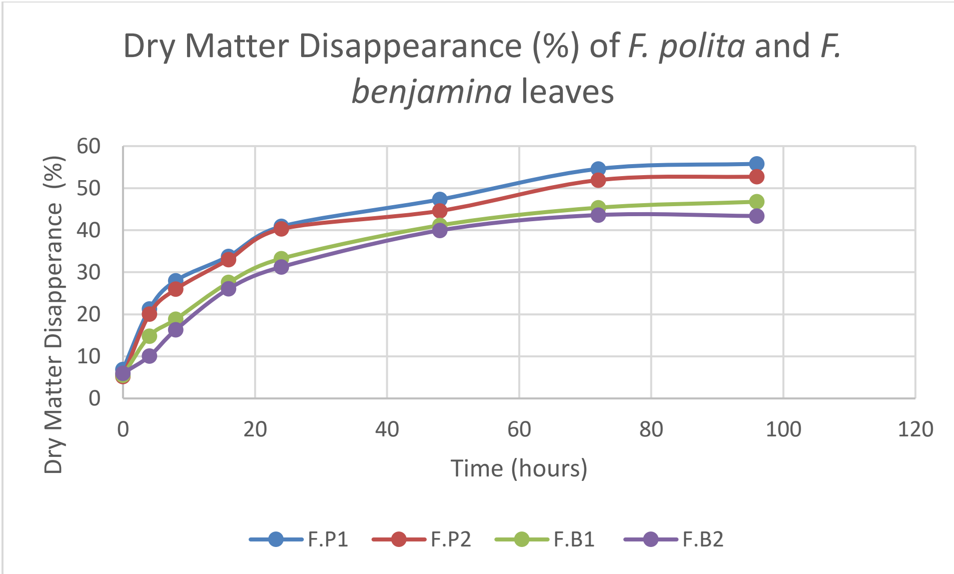

Figure 0.1. *In situ* dry matter disappearance (%) of *F. polita* and *F. benjamina* leaves harvested in summer and winter seasons.

F.P1: *F. polita* wet season harvest; F.P2: *F. polita* dry season harvest; F.B1: *F. benjamina* summer season harvest; F.B2: *F. benjamina* winter season harvest.

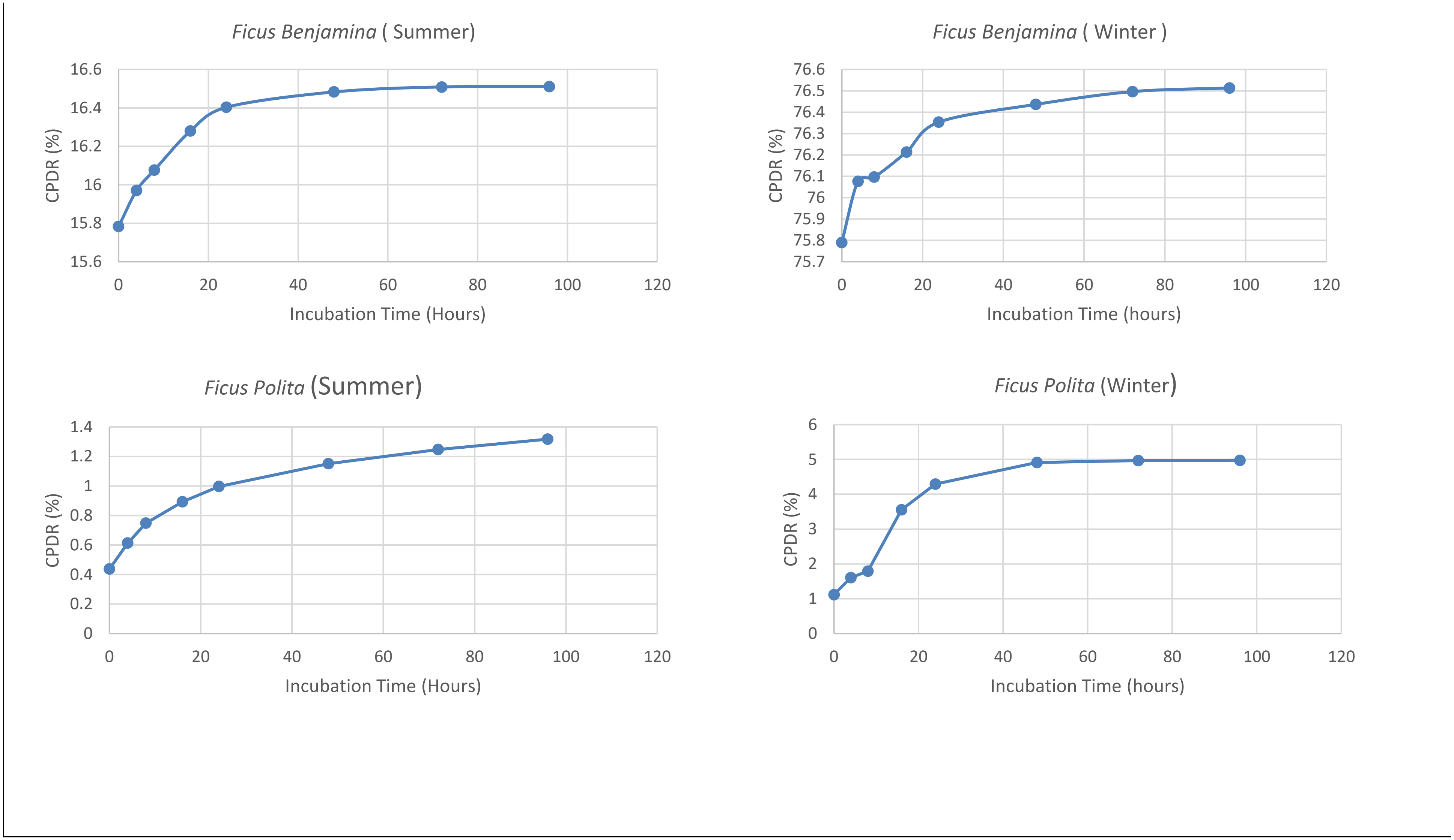

Figure 0.2. *In situ* crude protein disappearance (%) of F. polita and F. benjamina leaves in summer and winter seasons.

0

4.4: Discussion

4.4.1: *In situ* dry matter and crude protein degradability

Both browse samples had at least more than 5% readily soluble DM fraction (*a*). This was comparable to values obtained by Belachew *et al*., (2013) for the *Ficus sycomorus* species. Ficus species leaves are rich in organic compounds that include sugars, organic acids phenolics and other readily soluble compounds (Oliveira *et al*., 2009; Slatnar, *et al*., 2011; Arvaniti *et al*., 2019). These organic compounds ferment rapidly releasing instant energy for rumen microbiota which facilitate improved fiber degradability. Djouvinov and Todorov (1994) and Masama *et al*. (1997) reported a strong positive relationship between DM intake and microbial growth. As the period of incubation progressed, dry matter disappearance of the incubated samples increased due to the increase in rumen microbial degradation, displaying the Orskov and McDonald's (1979) trajectory. At 48 hours of incubation, the disappearance of dry matter from these *Ficus* species leaves harvested in the wet and dry seasons was above 40% of their potential degradability (PD).

Considering the typical mean retention time for fibrous feeds in ruminants (Kimambo and Muya, 1991), degradability after 48hrs of incubation is considered adequate to estimates rumen digestibility (Ehargavi and Ørskov (1987). All *Ficus* browse species had higher DM degradability characteristics in wet season than in dry season. The DM disappearance was lower during dry season than the wet season and is consistent with the findings of Aster *et al*., (2012) but contrary to that of Basha *et al*., (2015) who reported higher DM degradability during the dry season for browse species. The seasonal difference in DM degradability of selected *Ficus* browse species observed in the current study can be attributed to seasonal differences in accumulation of structural components, and differences in the stage of maturity. The findings of this investigation showed DM disappearance values for these *Ficus* species in wet and dry seasons after 48 hours of incubation to be satisfactory since they were above the prescribed 40 – 50% (Preston, 1986) to warrant further considerations as ruminant feed resources. The values were however lower than the 60% DM degradability at 24 hours for *Ficus sycomorus* and other browse tree leaves reported by Belachew *et al*. (2013). The disparity could reflect variations in environmental factors and stage of maturity (Imran *et al*., 2014; Ogunbosoye *et al*., 2015) that affect the chemical composition of the leaves (Anele *et al*., 2009), and antinutrients.

There was no difference in soluble fraction 'a' and outflow rate of degradation "c" of DM but *F. polita* had a higher ($P< 0.05$) insoluble but degradable "b" fraction and subsequently a higher DM potential degradability compared to *F. benjamina* in both seasons. The fraction 'a' represent rapid release of nutrients required for microbial protein synthesis (Costa *et al*., 2016). The values obtained for fraction 'b' of DM were within the range of 14-61% reported by Larbi *et al*., (1998) for *Ficus* browse forages. The low DM solubility recorded for these browse forages leaves suggests their ability to contribute a substantial amount of bypass nutrients to the hind stomach for the benefit of the animal (Njidda *et al*. 2016). The significantly higher effective degradability for *F. polita* compared to *F. benjamina* at rumen out flow rates of 0.02, 0.05 and 0.08 was probably due to its relatively higher hemi-cellulose and cellulose fractions which are degradable by the cellulolytic rumen microbes. According to Mupangwa (2003) variations in effective degradability of DM in forages closely correspond with the proportion of potentially degradable DM and level of NDF. Higher ADL content reduces ED (Haj-Ayed *et al*., 2000).

The fractional rate of degradation of the *Ficus* browse species in the study ranged between 45.5% and 52.1% in both wet and dry season and the rate at which feeds are digested in the rumen is as important as the extent of digestion as stated by Abegunde *et al*., (2011). Effective degradability (ED) of DM calculated at 0.02, 0.05 and 0.08 outflow rates from the rumen indicated that *F. polita* had higher values than *F. benjamina* in both dry and wet seasons. This might be due to chemical composition of these browse forages and could also be attributed to compositional differences of the browse forages, especially CP, fibre nature and concentration of polyphenolics and other anti-nutritional components (Salem *et al*., 2007). The chemical composition is largely affected by plant species, plant morphological fraction, environmental factors and stage of maturity (Salem 2005).

F. polita leaves had a significantly higher 'b' fraction compared to F*. benjamina* leaves but an opposite observation was made for the 'a' fraction in both seasons. The high soluble CP fraction of *F. benjamina* contributed much to the better potential degradability (PD), hence a higher ED compared to *F. polita* in all rumen outflow rates. Thus, a higher value obtained for CP potential degradability in the *F. benjamina* species in wet and dry seasons, may indicate a better nutrient availability for rumen micro-organisms in browsing ruminants. On the other hand, the lower soluble CP fraction in *F. polita* could be attributed to the amount of condensed tannins available in these species as these antinutrients reduce both the rate and extent of gas production (Frutos *et al*., 2002), with their suppressive effect on attachment of microbes to feed particles (McAllister *et al*., 1994) and a specific inhibition of ruminal degradation through an inhibition of micro-organisms growth and microbial enzymes activity (Waghorn, 1996; McSweeney *et al*., 2001). Previous reports suggested that the variation in the degradation parameters of the browse species may be due to the variation in chemical composition (Kamalak, 2006; Belachew *et al*., 2013;

Gusha *et al*., 2013). Tannins saponins and oxalates are known to decrease protein degradability by complexing with feed protein and have a capability to reduce the activities of rumen microbes and to interact with protein and carbohydrates (Salawu *et al*., 1997). In general, this reduction in protein degradation is associated with lower production of ammonia nitrogen and a greater non-ammonia nitrogen flow to the duodenum (Barry and Manley 1984; Waghorn *et al.*, 1994). Low CP degradability in these selected *Ficus* species leaves harvested in different seasons could be due to high ADL content attributed in the leaves (Table 3.1) because lignin acts as a mechanical barrier inhibiting microbial action (Van Soest, 1994), thus, rendering nutrient compounds unavailable during digestion (McDonald *et al.*, 2002).

These variations in PD of DM and CP in the rumen have been reported as a result of variations in fibre content levels (Gusha *et al*., 2013) or due to other factors such as ash content (Benjamin *et al*., 1995) or maturity (Kamalak, 2006; Gusha *et al*., 2013). In other studies, the results of the gas production and fermentation characteristics of the *Ficus* species suggested differences in nutritional value that is generally closely related to chemical composition (Cerrillo and Juarez, 2004; Kamalak *et al*., 2005; Salem, 2005) during wet and dry seasons. This high '*c'* value in both species indicates higher nutritive value of these browse forages and a potential to provide a significantly high rumen undegraded dietary nitrogen (RUDN) for digestion in the intestines especially at high rumen outflow rate. On the other hand, the ED of DM and N in *F. polita* and *F. benjamina* leaves calculated at an outflow rate of 0.02, 005 and 0.08 indicates that substantial amounts of the DM and N were degraded in the rumen, thus providing rumen degradable nitrogen (RDN) for microbial protein synthesis. However, the slowly degraded fraction of nitrogen could be providing valuable UDN to the intestine, with *F. polita* leaves in both wet and dry seasons providing more valuable UDN than *F. benjamina* leaves in wet and dry seasons.

4.5: Conclusion

Significant variation in *in situ* CP and DM degradability kinetics of *F. polita and F. benjamina* leaves among these two browse species and different seasons were observed. The results showed low DM and CP degradability of *F. polita* and *F. benjamina* leaves in both wet and dry seasons in the rumen which can be attributed to fibre concentration and antinutritional factors contained in these *Ficus* browse forages. Therefore, seasons have an effect in the ruminal degradability kinetics. Levels of CP degradability suggested the two browse species can be used as protein source for ruminants.

IN-VITRO POST-RUMINAL DIGESTIBILITY OF FICUS POLITA AND FICUS BENJAMINA LEAVES

Abstract

Nutrition is the major determinant of livestock production in the tropics. Protein supplements are required to improve the productivity of the livestock. The aim of the study was to determine the post ruminal *in-vitro* digestibility of DM and CP of *Ficus polita* and *Ficus benjamina* leaves harvested in two different seasons. Five trees from each species were randomly selected in the same area as they occurred along a linear transect of 1 km extending from west to east direction within Tshakhuma, Limpopo province, South Africa. The leaves were oven-dried, milled to pass through 1 mm sieve. Approximately 5 g of samples were placed in nylon bags (46 µm) and incubated in duplicates for 24 and 48 hour periods in the rumen of three cannulated Bonsmara steers. The residues were then analysed for DM and nitrogen (N). The *in vitro* DM and CP degradability of rumen undegradable residue collected after 24 and 48-hour incubation was determined by Pepsin-Pancreatin digestion procedure. Significant (P<0.01) species*season interaction was observed for $RDMD_{24}$. A higher (P>0.05) *in vitro* dry matter digestibility at 24-hr of incubation ($IVDMD_{24}$) for dry season compared to wet season was observed. The IVDMD for *Ficus polita* and *Ficus benjamina* leaves ranged between 5%-21.4%. The *in vitro* post ruminal CP digestibility of *F. benjamina* leaves in both wet (50.5% and 40.5%) and dry (26.6% and 17.4%) seasons was higher (*P* < 0.01) than that of *F. polita* (11.5% and 19.2%) in wet and (5.6% and 32.0%) in dry season, respectively in 24 and 48 hours of incubation, with significant species*season interaction for $RCPD_{24}$, $RCPD_{48}$, $IVCPD_{48}$ (P<0.01) and for $IVCPD_{24}$ (P<0.05). In conclusion, season and species affected the *in vitro* post ruminal digestibility of DM and CP of the browse species.

Key words: dry matter, crude protein, *in vitro* digestibility, *Ficus polita*, *Ficus benjamina*, browse, Pepsin-Pancreatin

CHAPTER FIVE

5.1: Introduction

Post ruminal digestibility is a measure of the availability of nutrients to the ruminant animal (Singh *et al*., 2010). Different methods of estimating post-ruminal nutrient availability from forages have been developed. The *in vitro* digestibility technique (Tilley and Terry, 1963) is ideal for rapid measurement of digestibility of large numbers of forage samples. The two-step procedure (TSP) is fast, low cost, and effective (Pires *et al.,* 2006) and ideal for measuring post ruminal endogenous enzymatic protein digestion. Salem *et al*. (2007) observed variation in leaf fermentation gas production among *Ficus* species which was attributed to compositional differences in browse forages, especially CP, fibre, nature and concentration of polyphenolics and other anti-nutritional components.

There is very little information on the comparative post ruminal (ileal) digestion of the leaves of *Ficus* browse species when harvested in different seasons. Such information is required for the harvesting and for the application of robust energy and protein feeding systems on a year-round-basis. The aim of the study was to determine the post ruminal *in-vitro* digestibility of DM and CP of *Ficus polita* and *Ficus benjamina* leaves harvested in two different seasons.

5.2: Material and method

5.2.1: Leaf sampling, sample preparation and chemical analyses

Leaves from *Ficus polita* and *Ficus benjamina* were sampled, harvested and the chemical components determined as described in chapter 3 and 4 respectively. The trial was carried out as described in chapter 3.

5.2.2: Ethical consideration

The study was approved by the Ethics Committee of the University of Venda (SARDF/19/ANS/05) as described in Chapter 4, under subsection 4.2.2.

5.2.3: Experimental design and Pepsin-Pancreatin digestion procedure

Treatments in this study were arranged as per the experimental design described in section 3.2.4. The nylon bags used to evaluate post ruminal digestion were the same as described in section. 4.2.6. The feed was milled through 1 mm screen and approximately 5 g was weighed and placed inside the nylon bags. Intestinal digestion was simulated using the TSP outlined by Gargallo *et al*. (2006). Rumen ungradable residues (RURs) recovered at the 24 and 48-hour of rumen incubation (section 4.2.3) were used. The RURs removed manually from the nylon bags were composited by treatment (species, season, incubation period and animal) and ground through a 1 mm sieve. Three nylon bags containing 1 g of RURs of each sample were placed in the glass bottles, which contained 750 mℓ of a 0.1 N HCl solution adjusted to pH 1.9 with 1 g $ℓ^{-1}$ of pepsin (P-7000; Sigma) and incubated for 1 hour in a water bath machine with constant horizontal movement at 39°C. After incubation, the bags were rinsed with tap water till runoff were clear, followed by a final rinse in deionized water before decanting into incubation bottles containing 750 mℓ of a pancreatin solution (0.5 mol $ℓ^{-1}$ KH2PO4 buffer adjusted to pH 7.75, containing 50 mg kg^{-1} of thymol to prevent bacterial growth and 3 g $ℓ^{-1}$ of pancreatin (P-7545; Sigma). Bags were incubated for 24 hours with constant horizontal movement at 39°C. After the pancreatic incubation, bags were rinsed with tap water until the runoff was clear, then finally rinsed with deionized water and dried at 60 °C for 48 hours. The recovered residues of 3 replicates in all the bags were composited by tree species, season of harvest, incubation hours and animal, then subsequently ground through a 1 mm sieve. Dry matter and crude protein losses were computed as the difference between the determined dry matter content of the incubated samples and the determined dry matter content of the in vitro digestibility residues.

5.2.4: Chemical analysis procedure for DM and CP

The residues were analysed for DM, and CP contents using the following methods:

RURs and *in-vitro* ungradable residues were dried in a forced air oven at 60°C for 48 h (AOAC, 1990; method 930.15) to determine DM content. The final residues in all bags were composited by the *Ficus* species treatment, incubation hour and steers and subsequently ground through a 1 mm sieve. The rumen degradable residues (RDR) were then analysed for N content using the Kjeldahl procedure (AOAC, 1990; method 984.13) and N was converted to CP as N x 6.25.

5.2.5: Statistical analysis

Data obtained from the quality of the leaves was subjected to analysis of variance (ANOVA) using the General Linear Model of MINITAB software version 17 (2014). Significant difference between the means was compared using the Tukey's procedure.

Statistical model:

$Y_{ij} = \mu + S_i + B_j + (SB)_{ij} + \varepsilon_{ij}$ Model 3

Where Y_{ij} = the observation of i^{th} plant species, and j^{th} season on DM and CP,

μ = overall mean common to all observations;

S_i = effect of the i[th] plant species,

B_j = effect of j[th] season on j[th] species,

$(SB)_{ij}$ = interaction between i[th] species and j[th] season,

ε_{ij} = random error

5.3: Results

5.3.1: *In vitro* dry matter digestibility

The ruminal degradability and *in vitro* digestibility data of DM of *F. polita* and *F. benjamina* leaves in wet and dry seasons from the nylon bags is summarized in Table 5.1. Significant species*season interaction was observed for $RDMD_{24}$. The DM degradability significantly increased as the period of incubation increased. *F. polita* had a significantly higher (P<0.01) dry matter degradability at 24-hour incubation ($RDMD_{24}$) than *F. benjamina* in both seasons. The post rumen dry matter digestibility doubled between the 24-h and 48-h incubation points. Effect of season was observed (P>0.05) for IVDMD. A higher (P>0.05) *in vitro* dry matter digestibility at 24-h of incubation ($IVDMD_{24}$) for dry season compared to wet season was observed. Species*season interaction between both species were not significantly different (P>0.05) except for $RDMD_{24}$ (P<0.01).

Table 0.1. Rumen Dry matter disappearance (%) at 24- and 48-hour incubation and subsequent post ruminal *in vitro* digestibility of *F. polita* and *F. benjamina* leaves harvested in two different seasons.

Species	Season	$RDMD_{24}$	$RDMD_{48}$	$IVDMD_{24}$	$IVDMD_{48}$
F. Polita	1	40.9[a]	45.3	5.2	19.2
	2	40.3[a]	43.6	11.9	21.4
F. Benjamina	1	33.3[b]	41.2	7.7	16.3
	2	31.3[b]	39.9	8.1	13.2
SEM		1.63	2.04	1.57	2.16

Species means				
F. Polita	40.6[a]	44.5	8.6	20.3
F. Benjamina	32.3[b]	40.6	7.9	14.7
SEM	0.82	1.02	1.11	1.53
Season means				
[1]Summer	37.1	43.2	6.5	17.7
[2]Winter	35.8	41.78	9.9	17.3
SEM	0.82	1.02	1.11	1.53
Significance				
Species	**	ns	ns	ns
Season	ns	ns	ns	ns
Species*Season	**	ns	ns	ns

**: $P < 0.01$; *: $P<0.05$; (ns) non-significant: $P >0.05$. $RDMD_{24}$: Dry matter degradability at 24 hours incubation; $RDMD_{48}$: Dry matter degradability at 48 hours incubation; $IVDMD_{24}$: *In vitro* dry matter digestibility at 24 hours of rumen incubation and $IVDMD_{48}$: *In vitro* dry matter digestibility at 48 hours of rumen incubation. [1]Summer: wet season; [2]Winter: dry season. [ab] Column means with different superscripts differ significantly at $P < 0.05$, SEM: standard error of means.

5.3.2: *In vitro* crude protein digestibility

The ruminal and *in vitro* digestibility of CP of *F. polita* and *F. benjamina* leaves in wet and dry seasons from the nylon bags is summarized in Table 5.2. Significant species*season interaction was observed for $RCPD_{24}$, $RCPD_{48}$, $IVCPD_{48}$ ($P<0.01$) and $IVCPD_{24}$ ($P<0.05$). The CP degradability of both *Ficus* browse species increased as the ruminal incubation period increased. *Ficus polita* leaves had significantly higher CP disappearance as well as CP digestibility at 24-hr and 48-hr than *F. benjamina* leaves at 24- and 48-h of rumen incubation in both harvest seasons. Species*season interaction between both species were significantly different. Effect of season was observed ($P<0.05$) for $IVCPD_{24}$ and ($P<0.01$) for $IVCPD_{48}$. A higher ($P<0.05$) *in vitro* crude protein digestibility at 24-h of incubation ($IVDMD_{24}$) for wet season compared to dry season was observed.

Table 0.2. Rumen Crude protein disappearance (%) after 24- and 48-hour incubation in the rumen and subsequent post ruminal *in vitro* digestibility of *F. polita* and *F. benjamina* leaves harvested in two different seasons.

Species	Season	$RCPD_{24}$	$RCPD_{48}$	$IVCPD_{24}$	$IVCPD_{48}$
F. Polita	1	16.2^{a}	17.8^{a}	11.5^{c}	19.2^{c}
	2	16.0^{b}	15.6^{b}	5.6^{c}	32.0^{c}
F. Benjamina	1	11.1^{c}	11.7^{c}	50.5^{a}	40.5^{a}
	2	10.7^{d}	11.0^{d}	26.6^{b}	17.4^{c}
SEM		0.49	0.54	2.20	1.47
Species means					
F. Polita		16.1^{a}	16.7^{a}	8.3^{b}	25.6
F. Benjamina		10.9^{b}	11.4^{b}	38.6^{a}	28.9
SEM		0.24	0.31	1.55	1.04
Season means					
[1]Summer		13.5^{b}	13.3^{a}	30.8^{a}	29.9^{a}
[2]Winter		13.6^{a}	14.8^{b}	16.1^{b}	24.7^{b}
SEM		0.24	0.31	1.55	1.04
Significance					
Species		**	**	**	ns
Season		**	*	**	*
Species*Season		**	**	*	**

**: *P* < 0.01; *P<0.05. $RCPD_{24}$: Crude protein degradability at 24 hours rumen incubation; $RCPD_{48}$: Crude protein degradability at 48 hours rumen incubation; $IVCPD_{24}$: *In vitro* crude protein digestibility after 24 hours of rumen incubation and $IVCPD_{48}$: *In vitro* crude protein digestibility after 48 hours of rumen incubation. [1]Summer: wet season; [2]winter: dry season. [abc] Column means with different superscripts differ significantly at *P* < 0.05, SEM: standard error mean

5.4: Discussion

5.4.1: *In vitro* dry matter digestibility

There is lack of information on the post ruminal IVDMD of these two *Ficus* browsable species. The $IVDMD_{48}$ values for *F. polita* recorded in this study were higher comparing with values observed in *F. benjamina,* which might suggest increased feed intake as the IVDMD and feed intake are positively correlated (Van Soest, 1994). However, high dry matter degradability and low dry matter digestibility in both *Ficus* species at 24 and 48 hours of incubation was recorded in wet and dry seasons; therefore, some of RDMD left will be able to reach the small intestines for absorption (Calsamigia and Stern, 1995). The IVDMD values for *F. polita* and *F. benjamina* leaves in wet and dry seasons observed in the present study range between 5%-21.4% which was less than the critical total digestibility threshold of 50% which is required for feeds to be considered as having acceptable digestibility or total digestibility (Owen and Jayasuriya, 1989), therefore, it appears that ADF and ADL fractions, which were significantly high, may have significantly lowered the IVDMD in these two selected *Ficus* species. SCA, (1990) indicated that, when IVDMD falls below 550 g/kg there is physical limitation on the rate of eating and the rate of digestion and passage through the gastrointestinal tract is restricted while live weight loss becomes inevitable.

Okunade *et al*., (2014) stated that the generally high IVDMD demonstrates the high nutritive potential of the browses when used in livestock feeding. It can be concluded that the DM digestibility of these browse species were affected by plant composition and the rate of digestion and passage through the gastrointestinal tract and also due to reduced uptake of essential nutrients from the soil and reduced photosynthetic activities of the plants induced by environmental stress during the dry season (Ahmed *et al*., 2017).

5.4.2: *In vitro* crude protein digestibility

There is lack of information on the post ruminal IVCPD of these two *Ficus* browsable species. The results observed showed high CP degradability of *F. polita* in both seasons than those of *F. benjamina* leaves in both time intervals. This can be due to forage quality and species which varies over the growing season and declines as the forage matures (Licitra *et al*., 1997) which causes an increase in the lignification of NDF. The results showed low crude protein degradability and high crude protein digestibility in both seasons for *F. benjamina* RUR's at 24 and 48 hours of incubation. High protein degradability was observed in the rumen and low protein digestibility for *F. polita* leaves in both seasons at 24-hr and 48-hr of incubation, suggesting a significant supply of rumen undegradable dietary protein for gastro-small intestinal digestion.

5.5: Conclusion

The study revealed that the IVDMD of these browse species was affected by season. The *F. polita* had a higher RDMD and IVDMD than *F. benjamina* in both seasons while the dry season browse had significantly higher $IVDMD_{24}$. It can be concluded that higher post rumen CP digestibility compared to the preceding rumen degradation suggests a high potential for RUDP supply which improve the efficiency of nitrogen utilization by ruminants.

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

6.1: General discussions

The aim of the study was to evaluate the potential of *Ficus polita* and *Ficus benjamina* leaves harvested in wet and dry season as supplementary protein sources for ruminant livestock in order to facilitate their optimum utilisation. The variation between the results of these two *Ficus* species could be due to the effects of species composition and morphological characteristics (Kilcher, 1981) as well as environmental differences. Interaction between season and species affected their nutritive value. The respective dry and wet season DM of *F. polita* content was within the previously reported range (295 – 305 g/kg DM), Abengunde *et al.,* (2011), while DM content of *F. benjamina* in both seasons was higher than the one reported by Bamikole, *et al.,* (2004). The lower ash content of *F. polita* leaves in both wet and dry seasons indicated that the plant has a comparatively higher organic matter constituent than *F. Benjamina* (Omoregie and Oluyemisi, 2010).The content of CP determined for these species in this study (136-207 g/kg DM) was higher than those reported by Bamikole, *et al.,* (2004) and Ndamitso *et al*., (2010). Browse species should contain crude protein (CP) content ranging from 30 g/kg - 260 g/kg during dry season (Raman 2003), the values observed in the present study were within this range. The high concentration of CP during dry and wet seasons in this study are high enough to be used as protein supplements to poor quality matured grasses during dry seasons to improve the productivity of ruminants.

The National Research Council (1989) recommends a minimum dietary ADF content of 19 to 21% and NDF content of 25 to 28% (dry matter basis) for lactating cows (NRC, 1989). NDF content in *F. Polita* was within the range of 670.0-880.0 g/kg DM that was reported by Abegunde *et al.,* (2011). The seasonal effect on the crude fibre content of *F. polita* was observed. Lack of information has been found regarding the chemical composition of these *Ficus* leaves harvested in different seasons. Dry seasons ADIN contents in these Ficus browse species are partially high than those of wet season because the proportion of nitrogen found as acid detergent insoluble nitrogen (ADIN) increases with the increasing forage maturity (Coblentz *et al*., 1998, Elizalde *et al.,* 1999 and Gonzalez *et al.,* 2001).

Minerals are required for nutrient metabolism and for proper functioning of organs. NRC (1976) recommended 3.0 g/kg as the critical level of Ca in the diet of ruminants. The Ca content of the selected *Ficus* browse species is high than the recommended by NRC (1975), thus making them to be good source of Ca. *F. benjamina* leaves in both seasons was observed to have higher Ca and Mg content than those of *F. polita.*

The K mineral content for *F. polita* leaves was recorded to be higher than *F. benjamina* K content in both seasons, therefore, this browse leaves had high K content when comparing to the recommended critical requirement level for grazing animals by Underwood, (1981). The results of this study indicates that *F. polita* leaves would not need K supplementation when they are fed to ruminants, while *F. benjamina* leaves would require K supplementation. Abdullah *et al*. (2013) recommended the range of 1.2 – 4.8 g/kg P for all classes of ruminants. In the current study, the P content level for *F. polita* is within the recommended range while that for *F. benjamina* leaves was lower than the recommended range. To meet the need of highly productive animals, forage should contain more than 1.5 g/kg of sodium (Na) and Na deficiency is more likely to occur in animals grazing on tropical pasture species and these species generally accumulate less Na than temperate species (Morris, 1980). Le Houerou (1980) also noted that all browse species are able at all their phenological stages to meet the energy requirements of livestock at maintenance level and often well above. Limited information has been found regarding mineral content of *F. polita* and *F. benjamina* leaves in different seasons.

The fractional rate of degradation of the *Ficus* browse species in the study ranged between 3 and 5 % in both wet and dry season. since the rate at which feeds are digested in the rumen is as important as the extent of digestion as stated by Abegunde *et al*., (2011). The results observed in this study for *F. polita* in dry and wet seasons are within the range of 2.8-7.1% reported by Abegunde *et al*, (2011). Effective degradability (ED) of DM calculated at 0.02, 0.05 and 0.08 outflow rates from the rumen indicated that *F. polita* had higher values than *F. benjamina* in both dry and wet seasons. This might be due to chemical composition of these browse forages and could also be attributed to compositional differences of the browse forages, especially CP, fibre nature and concentration of polyphenolics and other anti-nutritional components (Salem *et al*., 2007). The variations in PD of DM and CP in the rumen have been reported as a result of variations in fibre content levels (Gusha *et al*., 2013) or due to other factors such as ash content (Benjamin *et al*., 1995) or maturity (Kamalak, 2006; Gusha *et al*., 2013).

The $IVDMD_{48}$ values for *F. polita* recorded in this study were significantly higher comparing with values observed in *F. benjamina*. This could be the result of high fiber content among these *Ficus* browse species. This could be due to values high fibre content among the browse species. Low dry matter degradability and high dry matter digestibility in both browse species at 24 and 48 hours of incubation; therefore, less reaches the small intestines for absorption (Calsamigia and

Stern, 1995). The IVDMD values for *F. polita* and *F. benjamina* leaves in wet and dry seasons observed in the present study were less than the critical total digestibility threshold of 50% which is required for feeds to be considered as having acceptable digestibility or total digestibility (Owen and Jayasuriya, 1989), The in-vitro CPD results observed showed high CP degradability of *F. polita* in both seasons than those of *F. benjamina* leaves in both time intervals. This can be due to forage quality and specie which varies over the growing season and declines as the forage matures (Licitra *et al*., 1997) which causes an increase in the lignification of NDF. The results showed low crude protein degradability and high crude protein digestibility in both seasons for *F. benjamina* RUR's at 24 and 48 hours of incubation. High protein degradability was observed in the rumen and low protein digestibility for *F. polita* leaves in both seasons at 24-hr and 48-hr of incubation, suggesting a significant supply of rumen undegradable dietary protein for gastro-small intestinal digestion.

6.2: General Conclusion

This study showed that the DM and CP contents of the browse leaves evaluated on basis of their fermentation kinetics and *in vitro* digestibility, presented significant variations between *F. benjamina* and *F. polita* leaves harvested in wet and dry seasons. The study revealed that the interactions between season and species affected chemical composition, *in-situ* RDMD & RCPD and IVDMD & IVCPD values of these *Ficus* browse species. The browse leaves showed good nutritional quality in terms of their ruminal disappearance of CP. The CP content of *F. polita* and *F. benjamina* leaves in both seasons remained relatively high suggesting the possibility that leaves may be used as dry season fodder and protein source to poor-quality diets. It can be concluded that higher post rumen CP digestibility compared to the preceding rumen degradation suggests a high potential for RUDP supply, therefore leaving an opportunity to improve the efficiency of nitrogen utilization by ruminants.

6.3: Recommendations

It is recommended that *F. polita* and *F. benjamina* leaves in wet and dry seasons can be used as protein supplement to ruminants. Therefore, it is of importance to encourage farmers in communal areas wherein the livestock are adapted to browsing forage, to use *F. polita* and *F. benjamina* leaves as protein source during dry season.

Further research is necessary to study the following:

i. In vivo trials to assess the supplementary value of these browse species in animal response, in order to optimise the use of *Ficus* browse species as feed supplements for the dry season.
ii. Anti-nutritional factors in *F. polita* and *F. benjamina* leaves harvested in different seasons.
iii. Treatments to protect dietary protein from ruminal degradation.

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Appendix 1: Analysis of variance for chemical composition (g/kg DM) for *F. polita* and *F. benjamina* leaves harvested in two different seasons

Source	df	DM	Ash	CP	ADF	NDF	ADL	ADIN
Species	1	84144.3	17085.7	4582.5	31724.1	4129.2	35013.6	319.3
Season	1	3.3	102.1	3126.6	2385.7	253.9	2017.6	0.1
Species*season	1	1268.6	873.8	1696.9	6039.1	1004.7	3837.8	4.2
Error	8	1322.5	11.3	221.5	1319	6454.4	2188	14.5

**: $P < 0.01$; *: $P < 0.05$. df: Degree of Freedom, DM: Dry Matter, CP: Crude Protein, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre

Appendix 2: Analysis of variance for macro- (g/kg DM) and micro- (mg/kg) minerals of *F. polita* and *F. benjamina* leaves harvested in two different seasons.

Source	df	Ca	Mg	K	Na	P	Zn	Cu	Mn	Fe
Species	1	1984.0	12.2	468.8	0.24	1.14	154.1	126.8	40833.3	693602
Season	1	77.5	1.5	13.7	0.0	0.02	10.1	0.8	2241.3	114661
Species*season	1	31.0	0.9	0.03	0.02	0.0	60.8	0.8	3008.3	35534
Error	8	54.3	0.6	6.9	0.04	0.25	4.0	10.0	134.0	3437

**: $P < 0.01$; *: $P < 0.05$. df: Degree of Freedom, Ca: Calcium, Mg: Magnesium, K: Potassium, Na: Sodium, P: Phosphorus, Zn: Zinc, Cu: Copper, Mn: Manganese and Fe: Iron

Appendix 3: ***In situ*** **dry matter and crude protein disappearance (%) of** ***F. polita*** **and** ***F. benjamina*** **leaves harvested in two different seasons for interactions.**

	Season	0	4	8	16	24	48	72	96
Dry matter									
F. Polita	1	6.58[a]	21.24[a]	28.0[a]	33.79[a]	40.88[a]	45.3[a]	54.58[a]	65.79[a]
F. Polita	2	5.19[a]	20.04[a]	25.91[a]	33.03[a]	40.29[a]	43.59[a]	51.91[ab]	63.74[a]
F. Benjamina	1	5.48[a]	14.83[b]	18.85[b]	27.57[b]	33.25[b]	41.16[a]	45.36[b]	54.16[b]
F. Benjamina	2	5.99[a]	10.05[c]	16.32[b]	26.07[bc]	31.28[b]	39.96[a]	43.61[b]	51.38[b]
SEM		1.47	0.79	0.58	0.96	1.15	1.44	1.57	1.26
Significance		*	**	**	**	**	**	**	**
Crude protein									
F. Polita	1	0.79[c]	0.81[c]	0.59[c]	0.82[d]	0.81[d]	1.19[c]	1.98[c]	1.01[c]
F. Polita	2	3.05[c]	3.29[c]	3.29[c]	4.23[c]	4.17[c]	3.35[c]	3.04[c]	3.29[c]
F. Benjamina	1	16.51[b]	16.03[b]	16.02[b]	16.34[b]	16.40[b]	16.28[b]	16.19[b]	19.19[b]
F. Benjamina	2	76.55[a]	76.21[a]	76.15[a]	76.57[a]	76.39[a]	76.11[a]	76.5[a]	76.69[a]
SEM		1.11	0.85	0.76	0.41	0.32	0.80	1.07	0.92
Significance		**	**	**	**	**	**	**	**

**: $P < 0.01$; [ab] Column means with different superscripts differ significantly at $P < 0.05$; DM: Dry matter; CP: Crude protein; SEM: Standard error Mean

Appendix 4: Analysis of variance for degradability constants and calculated effective degradability at three passage rates for dry matter disappearance of ***F. polita*** **and** ***F. benjamina*** **leaves harvested in two different seasons for interactions.**

		Degradability	Constants			Effective	degradability	(%)
Source	Df	*a*	*b*	*C*	*a+b*	*K*=0.02	*K*=0.05	*K*=0.08
Species	1	0.17	74.2	0.0003	81.4	117.6	89.8	64.5
Season	1	0.7	0.7	0.000	2.6	5.9	4.3	3.0
Species*season	1	2.34	0.3	0.000	4.2	0.04	0.2	0.09
Error	4	4.29	2.83	0.0001	9.83	0.056	0.62	0.56

**: $P < 0.01$; df: Degree of Freedom; *a*: soluble fraction (%); *b*: insoluble but potentially degradable fraction (%); *c*: outflow rate of degradation (h^{-1}); ED: effective degradability; and *k*: rumen outflow rate (h^{-1}).

Appendix 5: Analysis of variance for degradability constants and calculated effective degradability at three passage rates for crude protein disappearance of *F. polita* and *F. benjamina* leaves harvested in two different seasons for interactions.

		Degradability constants				Effective degradability (%)		
Source	df	*a*	*b*	C	*a+b*	*K*=0.02	*K*=0.05	*K*=0.08
Species	1	4051.8	6.0	0.0008	3746.5	3834.6	3900.9	3936.4
Season	1	1841.5	5.0	0.00003	2038.2	1986.3	1945.1	1922.1
Species*season	1	1759.9	5.2	0.00001	1573.3	1620.8	1659.4	1681.2
Error	4	0.02	0.001	0.0002	0.01	0.02	0.02	0.02

**: $P < 0.01$; df: Degree of Freedom; *a*: soluble fraction (%); *b*: insoluble but potentially degradable fraction (%); *c*: outflow rate of degradation (h^{-1}); ED: effective degradability; and *k*: rumen outflow rate (h^{-1}).

Appendix 6: Analysis of variance for dry matter disappearance (g/kg) after 24- and 48-hour incubation in the rumen and then *in vitro* digestibility of *F. polita* and *F. benjamina* leaves harvested in two different seasons for interactions.

Source	Df	$RDMD_{24}$	$RDMD_{48}$	$IVDMD_{24}$	$IVDMD_{48}$
Species	1	138.7	30.31	1.54	92.9
Season	1	3.3	4.3	37.16	0.48
Species*season	1	1.0	0.13	31.23	20.38
Error	4	10.64	4.14	7.41	14.01

**: $P < 0.01$; df: Degree of Freedom, $RDMD_{24}$: Dry matter degradability at 24 hours incubation; $RDMD_{48}$: Dry matter degradability at 48 hours incubation; $IVDMD_{24}$: *In vitro* dry matter digestibility after 24 hours of rumen incubation and $IVDMD_{48}$: *In vitro* dry matter digestibility after 48 hours of rumen incubation.

Appendix 7: Analysis of variance for crude protein disappearance (g/kg) after 24- and 48-hour incubation in the rumen and then in vitro digestibility of F. polita and F. benjamina leaves harvested in two different seasons for interactions.

Source	Df	$RCPD_{24}$	$RCPD_{48}$	$IVCPD_{24}$	$IVCPD_{48}$
Species	1	54.66	56.33	2744.57	33.34
Season	1	0.03	4.25	649.4	80.25
Species*season	1	0.18	1.09	256.4	972.67
Error	4	0.0002	0.00	14.5	6.45

**: $P < 0.01$; df: Degree of Freedom, $RCPD_{24}$: Crude protein degradability at 24 hours rumen incubation; $RCPD_{48}$: Crude protein degradability at 48 hours rumen incubation; $IVCPD_{24}$: *In vitro* crude protein digestibility after 24 hours of rumen incubation and $IVCPD_{48}$: *In vitro* crude protein digestibility after 48 hours of rumen incubation