

CHEMICAL COMPOSITION, RUMINAL DEGRADABILITY AND *IN VITRO* DIGESTIBILITY OF DRY MATTER AND CRUDE PROTEIN OF *DICHROSTACHYS CINEREA* AND *BAUHINIA THONNINGII* LEAVES

BY

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DECLARATION

I, Mahwasane Mulalo Birgit, hereby declare that this dissertation is submitted in partial fulfilment of the requirements of the degree of Masters of Science in Agriculture (Animal Science) at the University of Venda. This dissertation is my own work and has not been submitted previously for any degree at this or any other university. It is original in design and in execution, and all reference material contained in this dissertation has been duly acknowledged.

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DEDICATION

To my father Alfred, mother Violet, brothers Thabelo, Takalani, Funanani, little sister Wangamulweli and my son Orilwela.

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ABSTRACT

Forage and browse legumes play an important role in sustaining livestock in small holder farming systems in the tropics, mainly as a result of their contribution to economic and environmental sustainability of livestock production. The study was conducted to determine the chemical composition, ruminal degradability and *in vitro* digestibility of dry matter (DM) and crude protein (CP) of *Dichrostachys cinerea* and *Bauhinia thonningii* leaves. The browse tree leaves were harvested in the wild in Shayandima, Limpopo province. The leaves were collected, oven-dried, milled to pass through a 1.0 mm sieve and analysed for chemical composition in the Animal Science Nutrition Laboratory, at the University of Venda. The browse tree leaves were analysed for DM nitrogen, neutral detergent fibre (NDF) and acid detergent fibre (ADF). Approximately 5 g of leaf sample milled to pass through through a 1 mm sieve were placed in nylon bags (external dimension: 6 × 12 cm, pore size of 41 µm) and incubated in duplicates for 0, 4, 8, 16, 24, 48, 72, 96 and 120 hours 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 outflow rate. The *in vitro* DM and CP degradability of rumen undegradable residue collected after 24 and 48 hour incubation was determined by sequential *in vitro* digestion in pepsin (abomasal) and pancreatin (small intestine) solutions. DM and CP content differed significantly ($P < 0.05$). *D. cinerea* leaves had higher levels of DM and CP content than *B. thonningii* leaves. DM and CP disappearance increased ($P < 0.01$) as the incubation period increased. There was no difference ($P > 0.05$) in soluble fraction ‘a’ and ‘b’ of DM of the two species. The CP components for both fraction ‘a’ and ‘b’ differed significantly ($P < 0.01$) for CP among the two species. There was significant difference ($P < 0.01$) in post-ruminal digestibility among the two species. CP digestibility of *B. thonningii* and *D. cinerea* leaves was reduced ($P < 0.01$). In conclusion, *B. thonningii* and *D. cinerea* leaves showed significant difference based on their fermentation kinetics and *in vitro* digestibility, suggesting a good nutritional quality which can be used as protein source for ruminants in dry season and supplement to low-quality diets.

Key words: dry matter, crude protein, degradability, *in vitro* digestibility, *Dichrostachys cinerea*, *Bauhinia thonningii*, Pepsin-HCL solution, browse trees

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LIST OF ABBREVIATIONS

<i>a</i>	Soluble Fraction
ADF	Acid Detergent Fibre
AOAC	Association of Official Agriculture Chemists
<i>b</i>	Insoluble but Potentially Degradable Fraction
<i>c</i>	Outflow Rate of Degradation per hour
Ca	Calcium
CP	Crude Protein
CPD	Crude Protein Degradability
CT	Condensed Tannins
Cu	Copper
DM	Dry Matter
DMD	Dry Matter Degradability
DMI	Dry matter intake
ED	Effective Degradability
Fe	Iron
<i>g</i>	Grams
<i>g/kg</i>	Grams per Kilogram
<i>g/kg DM</i>	Grams per Kilogram Dry Matter
IVDMD	<i>In vitro</i> Dry Matter Digestibility
IVCPD	<i>In vitro</i> Crude Protein Digestibility
K	Potassium

Mg	Magnesium
mg/kg	Milligram per Kilogram
N	Nitrogen
Na	Sodium
NDF	Neutral Detergent Fibre
NE _m	Net energy maintenance
OM	Organic Matter
P	Phosphorus
RUP	Rumen Undegraded Protein
RUR	Rumen Undegraded Residues
SEM	Standard Error Mean
TDN	Total digestible nutrients
Zn	Zinc
Mn	Manganese

CHAPTER 1: INTRODUCTION

1.1. Background

The main feed resources for livestock in small-scale farmers are natural pastures and crop residues which are low in both quantity and quality, (< 8% Crude Protein (CP), (Yavuz, 2007) for sustainable animal production. The low quality and quantity of natural pastures results in low growth rate, poor fertility and high mortality rates of ruminant animals (Odongo *et al.*, 2002; Shem *et al.*, 2003). Dietary energy for drought power animals is limited by the nutritional status of livestock and thereby affecting crop production. Livestock production could be increased by cultivating and establishing high quality forages containing 10 – 30% CP, with high yielding ability and that are adapted to the environment. There has been a drastic increase in the cost of feed in recent years owing to the economic hardship faced by most African countries (Tshabalala *et al.*, 2013). Only a few communal farmers can afford to buy supplementary feed for livestock.

An interesting challenge for scientists in the field of animal nutrition is the introduction of alternative feedstuffs that could be obtained from adverse environments (Vasta, 2008). However, some of these alternative feed resources contain secondary compounds, such as condensed tannins that, which when present in high concentration in the diet can negatively affect animal productivity (Makkar, 2003; Min *et al.*, 2003). There is need to introduce the use of browse species such as *Dichrostachys cinerea* and *Bauhinia thonningii* as alternative feed resources.

Dichrostachys cinerea (Sickle bush) is a thorny, semi-deciduous leguminous tree growing up to 8 m tall. It has bipinnate leaves, 4 to 8 cm long, 5 to 15 pairs of pinnae, each with 12 to 30 pairs of leaflets. *Bauhinia thonningii* (Camel's foot) is a tree legume of about 4 to 15 m high with a round crown. The leaves are glossy, bio-loped, reticulated, 15-17 cm long (Hauze *et al.*, 2016). The leaves of *Bauhinia thonningii* look like camel's foot and account for the tree is common name "camel's foot". The pods of *B. thonningii* contain an edible pulp and pea-like seeds.

Bauhinia thonningii and *Dichrostachys cinerea* are both multipurpose trees and with their pods and leaves are consumed by livestock. Livestock eat leaves in wet months of November to February because they are not hard with serrated margins. In Sudan, roasted seeds of *D. cinerea* are consumed by humans (Baumer, 1983). Animals do not compete with human for food since we do not utilize seeds for human consumption in South Africa. The use of forage

and browse legumes as an alternative protein source is currently examined. Some nutritional characteristics such as voluntary intake and effects of anti-nutritional factors still need to be investigated.

1.2. Problem statement

Farmers in the tropics face a critical shortage of animal feed particularly during dry season. Vhembe District is a drought prone area and farmers struggle to get feeds for animals especially during winter and this reduces animal production levels. Shortage of quality feeds is the major constraint in livestock production, particularly under smallholder farmers during the dry season in Vhembe District. Grasses are normally dry with a crude protein (CP) content ranging from 1.5% in dry season to 10% in wet season (Raman, 2003). The natural pastures, which the animals feed on during the dry seasons, are low in protein content and high in fibre and lignin content which result in low feed intake (Baloyi, 2002). The digestibility of grass rapidly declines with maturity leading to a decline in the body condition of livestock. Browse trees such as *D. cinerea* and *B. thonningii* can grow in a wide range of well-drained soils and are drought tolerant. Knowledge of how the chemical and nutritive value of browse plants and plant parts that are utilized by livestock during dry season is indispensable among communal farmers.

1.3. Justification of the study

There is limited published information regarding the ruminal degradability and *in vitro* digestibility of *D. cinerea* and *B. thonningii* leaves. There is limited published information on the nutritive value of *B. thonningii* leaves as a source of protein for intensively fed cattle; moreover, less is known on the secondary compounds suppressing animal production for the selected browse species. However, some information is available on the chemical composition of *D. cinerea*. There is need to analyze the nutritional value of browse species that could be used as a protein source for animals and give useful information to local farmers to improve their livestock production systems.

Improving the feed resource base by identifying alternative and more nutritious feeds is necessary to alleviate the prevailing nutritional problems of livestock in Vhembe District. Improvement in the availability and nutritional quality of feed resources can be achieved through

the identification of high quality browse species of high yield potential that are adapted to local conditions, such as *D. cinerea* and *B. thonningii*. Establishing these browse species in local environment for small-scale farmers will provide information to them which will help in improving the production value of animals which are becoming less productive due to insufficient nutritional feeds and lack of knowledge.

The objectives of the present study were to determine the chemical composition, ruminal degradability and *in vitro* digestibility of dry matter and crude protein of *B. thonningii* and *D. cinerea* leaves.

1.4. Objectives

The main objective of the study was to determine the nutritional value of browse leaves as protein sources for ruminant livestock.

The specific objectives of the study were to determine the:

- i. Chemical composition of *D. cinerea* and *B. thonningii* leaves
- ii. Dry matter (DM) and protein degradability of *D. cinerea* and *B. thonningii* leaves, and
- iii. *In vitro* digestibility of dry matter and crude protein of *D. cinerea* and *B. thonningii* leaves

1.5. Hypotheses

Null hypothesis

- i. There is no difference in the chemical composition of *D. cinerea* and *B. thonningii* leaves,
- ii. There is no difference in ruminal degradability of DM and protein of *D. cinerea* and *B. thonningii* leaves
- iii. There is no difference in *in vitro* digestibility of DM and CP of *D. cinerea* and *B. thonningii* leaves

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Poor nutrition is one of the major constraints of animal production in Vhembe District of South Africa. This is because animals live predominantly on high fibre feeds, which are often deficient in nutrients (nitrogen, minerals, etc.). Animal feed supply quantity and quality is aggravated in arid and semi-arid tropical regions by erratic and unreliable rainfall that limits biomass yield and growing seasons of herbaceous species (Melaku, 2010). These leads to livestock being exposed to indigenous browse species when all annual grass and herbaceous species has died out. Leguminous browse species fix atmospheric nitrogen, and this contributes to the increased crude protein (CP) content of *Dichrostachys cinerea* and *Bauhinia thonningii* (Melaku, 2010). In areas of low rainfall, the leaves of browse are regarded as important feed for the nutrition of grazing animals because browse provides supplement of protein and energy when herbaceous species has died out and also used as alternatives that could be utilized during drought or winter season. Leaves of leguminous browses provide a good source of protein supplement to ruminants in tropical Africa (Ngwa *et al.*, 2001).

2.2. Feed resources and utilization in Vhembe District

Livestock feed resources in Vhembe District is mainly natural grazing, browse, pasture and crop residues. However, there is a decline in the size of the grazing land and degradation through overgrazing, the expansion of arable cropping and cutting of household stands. The feeding systems include communal or private natural grazing and browsing, cut and-carry feeding, hay and crop residues. The availability and quality of forage are not available year round. As a result, the production made in the wet season are totally or partially lost in the dry season. Inadequate amount of feed during the dry season is a major cause for declining in the productivity of ruminants (Amole and Ayantunde, 2016).

Hay making is commonly used as a means of feed preservation technique in Vhembe District, which is expected to mitigate problems of livestock feed shortage during the dry period and therefore such experience is a good indicator that there are certain practices of efficient feed utilization (Alemayehu, 2003). Some improvements on handling and preservation are important

to make more quality hay. High quality hay can be defined as forage that is dried without deterioration and retaining most of its nutrients (Greenham *et al.*, 2007).

The quantity of different crop residues produced depends on the total area cultivated, the season's rainfall, crop species as well as other inputs such as fertilizers (Daniel, 1988). Although it is neither quantitatively nor qualitatively adequate to support profitable animal production (Alemu *et al.*, 2013) grazing is also the predominant form of ruminant feeding system in most parts of the extensive and smallholder crop-livestock farming areas in Vhembe District. Furthermore, the feed for livestock arising from natural pasture fluctuates considerably in quality components as protein and fiber which are generally inversely proportional to each other (Tohill, 1987).

2.3. Nutrient requirement of grazing ruminants

Profitability and production efficiency are common objectives for many beef cattle producers. Feed represents the majority of total costs associated with livestock production. It is important to understand the biological priority for nutrients by beef cows. Nutrients will first be used for maintenance of the animal. The second priority is growth, followed by milk production, and finally reproduction. Beef cows have different requirements based on their stage of production. The nutritional requirements in each stage must be met to achieve optimal cow health production. The nutritional requirements based on their stages of production for mature cows and heifers are presented in Table 2.1.

Table 2.1. Nutrient requirements based on stages of production for mature cows and heifers

Stage of production	Required nutrient diet density	DMI (kg)	%TDN	NEm (Mcal/d)	%CP
Pre-Calving	Mature cows	10.5 - 12	54.6	15 - 16.5	8.6
	1 st calf heifers	10 - 11.5	58.3	16 - 18	9.0
Post-Calving	Mature cows	12 - 13.5	59.2	19.20	10.5
	1 st calf heifers	10 - 12	60.6	19.5 - 21	10.5
Lactating and pregnant	Mature cows	11 - 12	55.1	15 - 16	8.7
	1 st calf heifers	10 - 12	57.0	15.5 - 16.5	8.9
Mid-Gestation	Mature cows	9.5 - 11.5	47.4	9 - 10	6.6
	1 st calf heifers	9 - 11.5	50.9	10 - 11.5	7.3

DMI: Dry matter intake, TDN: Total digestible nutrients, NEm: Net energy maintenance, CP: Crude protein

(Source: Lunn, 2013)

The amount of protein a cow needs depends on her size, growth, milk production and stage of pregnancy (Moran, 2005). However, milk production is the major influence on protein needs. The amount of crude protein requirements at different stages of lactation are presented in Table 2.2.

Table 2.2. The amount of crude protein requirements at different stages of lactation

Milk production	Crude protein requirement (g/kg DM)
Early lactation	160 – 180
Mild-lactation	140 – 160
Late lactation	120 – 140
Dry	100 – 120

(Source: Target 10, 1999)

Cows need a certain amount of fibre (Table 2.3.) in their diet to ensure that the rumen functions properly to maintain the fat test. The levels of fibre that cows need in their diet are presented in Table 2.3.

Table 2.3. The minimum amount needed in cow's diet for healthy rumen function using three different measures of fibre.

Fibre measurement	Minimum amount of dietary fibre (g/kg DM)
Neutral detergent fibre	300
Acid detergent fibre	190
Crude fibre	170

(Source: Target 10, 1999)

2.4. Productivity and nutritive value of browse species

2.4.1. Productivity/availability

Browse species are perennial plants, fodder trees are not susceptible to sudden climatic changes and continue to produce high quality fodder even during drought years when grasses and other annual forages are dry and long gone (Paudel and Tiwari, 1992). Their capacity to grow fast enables them to produce large quantities of biomass, which can be used not only for animal feeding but also as mulch in cropping systems. They are also used to control soil erosion (Sibanda, 1993). When intercropped with food crops, fodder legumes do not compete with food crops for nutrients as their deep root system enables them to tap nutrients from the deeper soil layers, which are generally not available for shallow rooted food crops. They also improve soil fertility by fixing atmospheric nitrogen and have other symbiotic relationships, which enhances uptake of minerals such as phosphorus by plants (Topps, 1992). In the dry season, fodder trees also provide shade to animals and protect them from the hot and dry weather conditions which are common at that time of the year. The nutritious profusion and perennial performance of browses afford round the year provision of forage for livestock (Mtengeti and Mhelela, 2006). Forage quality decreased from wet to dry season with greater declines in grasses than browse (Mpinyane *et al.*, 2015), that is why cattle browse when the leaves are young in September and stop eating them in December when the leaves are hard with tough, serrated margins and therefore unpalatable (Aganga and Tswenyane, 2003).

2.4.2. Nutrient content of browse leaves

Nutritive value describes the forage's capacity to meet the animal's nutritional needs. This value depends on the quantity of feed which is digested and absorbed and the amount of essential nutrients (protein, fat, carbohydrates, minerals and vitamins) which it contains. A good nutritive value description of forages should include measures of voluntary intake, nutrient or anti-nutrient content, digestibility and metabolism of the nutrients. Fodder trees are important sources of high quality feed for grazing ruminants and as protein supplements to improve the productivity of herbivores fed on low quality feeds (Fabian, 2013). Characterization of forages for ruminants, include the determination of fibre, lignin and protein contents and critical to these is an accurate DM determination (Givens *et al.*, 2000). Forage is generally limited in yield in winter as compared to summer and, in ruminants, the low quantity of available browse may not be sufficient to satisfy their nutritional and energy requirements, especially in high population density (Torbit *et al.*, 1985). Many trees produce potentially nutritious fruits containing up to 200 g/kg CP (Tanner *et al.*, 1990). Browsers contain crude protein content ranging from 30 g/kg DM to 260 g/kg DM during dry season (Roman, 2003). Most browsers 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). Legume forages have high nitrogen content and can be used as nitrogen supplement in ruminant's diet (Baloyi *et al.*, 2001) and browsable species are highly nutritious and contribute substantially to the diet of livestock in communal areas (Fabian, 2013). Shrubs often retain high levels of nutrients during the dry season increasing with the first flush of new growth before it rains (Aganga *et al.*, 2005). The forage quality can be affected by palatability, intake, digestibility and anti-nutritional factors (Lacefield, 2005).

Chemical component can provide important biochemical information, leading to a better understanding of factors that may limit animal performance. Browse species are known to be high in nutritive value, with low variation over time compared to grasses (Fadel *et al.*, 2002). *Dichrostachys cinerea* contained from 73.82% DM, 5.55g/100g Moisture, 6.54% \pm 0.001 Ash, 13.37% \pm 0.004 CP, 55.38% \pm 0.96 NDF, 33.70% \pm 0.86 ADF (Aganga *et al.*, 2005). *Dichrostachys cinerea* (g/kg) have 172.7 \pm 9.4 CP, 270.3 \pm 12.2 ADF and 452.8 \pm 13.7 NDF (Madibela *et al.*, 2004). Most of the plants have CP content around 80 g/kg DM in the dry season and ash ranged from 32.5 – 95.8 g/kg DM, and can still retain up to 50% moisture in the dry season (Aganga *et al.*, 2005). *Dichrostachys cinerea* leaves (g/100g) were having 5.5 Moisture, 14.48 Ash, 16.64 CP, 18.50 CF (Fabian, 2013). *D. cinerea* has relatively high protein

content (90 – 190 g/kg DM) and may be used as an alternative source of CP in cattle fattening meals (Choongo *et al.*, 2008). *Bauhinia thonningii* leaves (%) had 93.25 DM, 5.93 Ash, 8.58 CP, 40.64 NDF and 17.06 ADF (Chibinga and Nambeye, 2016). Ighodaro *et al.* (2012) observed 10.09 CP and 6.10 Ash for *B. thonningii* leaves. *Bauhinia thonningii* leaves (%) were having 95.4 DM, 4.0 Ash and 20.0 CP (Tijani *et al.*, 2012).

2.5. Effects of climatic change and rising temperatures on browse species

Browse availability is important for livestock in small-scale farming since it enables survival rate of livestock during dry season and drought years. The effects of climate change include, higher temperatures and changing in rainfall patterns. As temperature and carbon dioxide (CO₂) levels change, optimal growth ranges for different plants also change; species alter their competition dynamics, and the composition of mixed grasslands changes (Fabian *et al.*, 2013). For example, higher CO₂ levels will affect the proportion of browse species. Rising temperatures increase lignifications of plant tissues and thus reduce the digestibility and the rates of degradation of plant species, and the resultant reduction in livestock production due to low quality feed may influence the food security and incomes of smallholder farmers (Fabian *et al.*, 2013).

2.6. Mineral content in browse species and mineral requirement in ruminants

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). High yielding cows require much more dietary calcium (Ca) and phosphorus (P) than low yielding cows because of the richness in milk (McDowell *et al.*, 2012). A number of macro and micro elements have been shown to be essential for animals (NRC, 2001).

Forages provide an important source of minerals to ruminants in preventing diseases as well as inhibiting or stimulating ruminal microbial activity (Underwood, 1981). However, they are deficient in one or more minerals and supplementation become the solution for optimal animal performance and health. Mineral concentrations are affected by climatic conditions (Ramirez *et al.*, 2005). Sulphur deficiency, like protein, depresses microbial activities in the rumen (Whiteman, 1980). However, legumes are more efficient at extracting calcium from soils low in calcium. Phosphorus levels in browse is at the greatest during periods of active growth and then declines rapidly and levels off as senescence is approached (Jones and Weeks, 1985).

As many as 14 different minerals are required by ruminants to maintain sound health and production. Some are required in relatively large amounts and form a significant proportion of the body. Such minerals are often classed as macro minerals (Calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K) and sodium (Na)). Others are required in much smaller amounts are known as micro minerals or trace elements (zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe)). *Dichrostachys cinerea* leaves contained 13.5 g/kg Ca and 1.5 g/kg P, while *B. thonningii* had 17.6 g/kg Ca and 1.9 g/kg P (Chibinga and Nambeye, 2016). The mineral contents observed by Aganga and Mesho (2008) were 12.7 g/kg Ca, 4.2 g/kg Mg, 12.8 g/kg Na, 3.05 g/kg P, 17.1 g/kg K, 800 mg/kg Fe, 300 mg/kg Mn and 10 mg/kg Zn for *D. cinerea* leaves. Mineral contents for *B. thonningii* were 17.4 g/kg Ca, 4.3 g/kg Na, 2.93 g/kg Mg and 7.8 g/kg K, 262.5 mg/kg Fe, 44.5 mg/kg Mn, 25.1 mg/kg Zn, 11.9 mg/kg Cu (Ighodaro *et al.*, 2012).

2.7. Limitations to the use of forage legume as feed resource for animals

Forage legumes are good protein sources for grazing animals but the presence of anti-nutritional factors suppress the ruminant production (Min *et al.*, 2003). The main limitation to effective utilization of fodder legumes as feed for ruminants is the high content of tannins and other anti-nutrients such as saponins, cyanogens, mimosine, coumarins, etc which limit nutrient utilization (Leng, 1997; Makkar, 1993). These compounds are also known to have other detrimental effects, which may range from reduced animal performance to neurological effects and increased animal mortality rates (D'mello, 1992). The toxic effects of these compounds depend on their concentration in a fodder species and level of intake by animals. The most widely occurring anti-nutrient in plants is a group of polyphenolic compounds commonly called as tannins. Tannins limit animal performance by suppressing intake and digestibility of forages (Meissner and Paulsmeier, 1995). They bind feed proteins and enzymes to form feed protein-

tannin complexes, which are resistant to both rumen microbial and enzymatic degradation. They also lower enzyme activity (Aufrere *et al.*, 1995).

2.8. Anti-nutritive factors found in browse tees

Utilization of nutritive value potential from browse legume fodder is limited by the presence of anti-nutritive factors such as phenolics and tannins (Kumar and D'Mello, 1995). Tannins are a complex group of polyphenolic compounds found in wide range of plant species commonly consumed by ruminants (Frutos *et al.*, 2004). They are conventionally classified into two major groups: hydrolysable and condensed tannins (proanthocyanadin), wherein their effect can either be beneficial or harmful depending on the type of tannins consumed. Forages with high (over 10% of DM) dietary condensed tannins are often associated with low palatability, low intakes and poor performance, whereas forages with medium (4 -10% of DM) dietary concentration of condensed tannins have an effect of low dietary N (Waghorn *et al.*, 1997). The effects of polyphenolics on the nutritive value of legumes in ruminants may vary from merely affecting the species composition of the microflora in the rumen to complexing with protein, carbohydrates and minerals and thus reducing or completely preventing their availability in the rumen and postruminally (Baloyi *et al.*, 2001). Tannin content of most plants ranged from 1.03 - 2.98% (Aganga *et al.*, 2005). Medium condensed concentration of condensed tannins in forages can be used to improve the efficiency of N digestion in ruminants grazing on fresh forage diets (Min *et al.*, 2003). Tannins can prevent bloat and suppress internal parasites (Hoste *et al.*, 2006).

Negative effects of tannins are loss of appetite, decrease in degradation and induced digestive responses (Aganga *et al.*, 2003). There were more condensed tannins in mature leaves (41.2 g/kg⁻¹) than young leaves (39.9 g/kg⁻¹), that is why cattle browse *Brachystegia spiciformis* when the leaves are young in September and stop eating them in December when the leaves are hard with tough, serrated margins and therefore unpalatable (Baloyi *et al.*, 2006). Where the tannin-protein complexes are associated with the low pH of the abomasum, an additional source of protein is made available for absorption by the animal species which contain some tannins. This provides both degradable and undegraded rumen N and is a more effective source of supplemental N for ruminants. Tannin-containing feeds cause adverse effects on the meat and milk fatty acids composition, depending on the amounts of condensed tannins present in the diet (Vasta *et al.*, 2008).

2.9. Rumen Degradability of browse species

When obtaining the protein value of the feed for ruminants, expressed as amino acids truly absorbed from the small intestine, which is now common to the new protein evaluation systems (Givens *et al.*, 2000), involves measurements of several characteristics of the feed (Madsen *et al.*, 1995). These characteristics are related to the fate of the protein in the feed during its passage through the digestive tract and to the energy- yielding process in the rumen which drive microbial protein synthesis in the rumen. Factors related to the feed protein are degradability of the feed protein in the rumen, content of amino acids in the undegraded crude protein, and finally, small-intestinal digestibility of the amino acids in undegraded dietary protein.

The principle of the *in situ* method is the incubation of small feed samples in the rumen in fibre bags (Givens *et al.*, 2000). The bags have pores small enough to retain the feed sample, but large enough to allow bacteria to enter the bags. Due to the small amounts of feed sample incubated, the feed under examination will not affect the rumen fermentation, and it is assumed that the conditions within the bag are similar to the conditions in the surrounding rumen content. *In situ* techniques for the estimation of protein and degradability in the rumen and intestinal digestibility of rumen undegraded dietary protein require access to cannulated animals (Givens *et al.*, 2000).

The rate and extent of fermentation of DM in the rumen are very important determinants for the nutrients absorbed by ruminants (Kamalak *et al.*, 2005). Reduced rumen protein degradability due to the presence of condensed tannins limits the supply of rumen ammonia for microbial activity. This, in turn, negatively affects the utilization of poor quality cereal crop residues which are a major component of ruminant livestock diets in semi-arid areas (Mlambo, 2009). High degradability values after 48 hours imply high digestibility since degradability values at this time are regarded as being equivalent to digestibility (Ehargava and Ørskov 1987). The leaves of the browse forages showed high potential as a feed supplement to animals in semi-arid areas especially in terms of crude protein supply for effective microbial activity in the rumen (Njidda *et al.*, 2012). The inclusion of highly digestible legumes such as birdsfoot trefoil in pasture plantings can increase the productivity of grazing livestock because forage legumes produce their own nitrogen as long as they are inoculated with the proper *Rhizobium* bacterium at planting. Plants can meet their own nitrogen fertilization needs as well as those associated with pasture grasses Jennifer (2013).

Protozoal and bacterial populations in the rumen tended to increase with the increasing level of leaf meal, especially the protozoa population (Mon Seng *et al.*, 2001). In ruminants fed with high quality fresh forage diets (25 – 35g N/kg and 10 – 11MJ of ME/kg DM) most proteins are rapidly solubilized and release between 56 and 65% of N concentration in the rumen during mastication; consequently, large losses of N occur (25 – 35%) as ammonia absorbed from the rumen (Min *et al.*, 2000). The protein content of forage tree legume leaves (12 – 30%) is usually high compared to that of natural grasses (3 – 10%) (Goodchild A, 1994). Feeds containing less than 1.3% N (8% crude protein) are considered deficient as they cannot provide the minimum ammonium levels (45 mg N/1) required. However, condensed tannins in some tree legumes form complexes with plant, proteins which decrease their rate of degradability in the rumen, thereby decreasing rumen ammonia concentrations and increasing the amount of plant protein bypassing the rumen. High degradability was found in all species which did not contain tannins, while most tannin-containing species were of low degradability (< 39%) (Godchild 1994), because condensed tannins depress degradability of feed in the rumen by affecting the activity of enzymes involved in carbohydrate and protein breakdown.

2.10. *In vitro* digestibility of browse species

Nutrient digestibility in feeds represents the amount of nutrients in a feed actually accessible to the animal (McDonald *et al.*, 2011). Digestibility is considered as the most essential factor in evaluating the nutritional quality of feed (McDonald *et al.*, 2011). The mobile bag technique is usually used to measure crude-protein digestibility, but the values are also assumed to represent amino acids digestibility, as amino acids digestibility is the value required in the modern protein evaluation systems. In studies by Weisbjerg *et al.*, (1996), which included 15 different concentrates, it was shown that the total amino acid digestibilities were similar to crude protein digestibilities. Large differences in amino acid digestibilities were observed among different concentrates, with the highest for protamyl (0.98) and the lowest for the cottonseed cake (0.84) (Weisbjerg *et al.*, 1996).

The *in vitro* three-step procedure by Gargallo *et al.* (2006) mimics post ruminal digestive processes by sequential digestion in pepsin (abomasal digestion) and pancreatin (small intestine digestion). This is achieved by estimating the nutrient digestibility in abomasal and intestinal digestion using pepsin and pancreatin, respectively, from ruminal degradability residues (Gargallo *et al.*, 2006). 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.

Tree forages with a low NDF content (20 – 35%) are usually of high digestibility and species with high lignin contents (>29%) are often of low digestibility (Goodchild, 1994). Values between 0.80 and 0.85 are adopted for the true digestibility of protein from temperate foods with microbial amino acids (ARC, 1984: van Bruchem *et al.*, 1989). Lignin content of tree foliage was negatively correlated ($r = - 0.92$) with feed digestibility in nylon bags (Baumualin *et al.*, 1980).

2.11. Summary

Indigenous multipurpose species are well adapted to the environment and contribute substantially to the diet of livestock throughout the year. Browse fodder is a potentially inexpensive, locally produced protein supplement for ruminants particularly during the periods of greatest deficit when the quantity and quality of herbage is limited. Feed supply and quality are major constraints experienced by small-scale farmers during dry season. *Bauhinia thonningii* and *D. cinerea* have been shown to be high quality browse species which can supply essential nutrients to the animals (Nassoro, 2014).

CHAPTER 3: DETERMINATION OF CHEMICAL COMPOSITION OF *BAUHINIA THONNINGII* AND *DICHROSTACHYS CINEREA* LEAVES

Abstract

Browse legumes are generally good sources of energy and protein for grazing ruminants. The high protein content of these legumes suggests that they have high potential for use as protein supplements in ruminant feeding. The objective of the current study was to determine the chemical composition of *Dichrostachys cinerea* and *Bauhinia thonningii* leaves. The browse tree leaves were harvested in the wild in Shayandima, Limpopo province. The leaves were collected, oven-dried, milled to pass through 1.0 mm sieve and analysed for dry matter (DM) and crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) in the Animal Science Nutrition Laboratory, at the University of Venda. *D. cinerea* leaves had higher levels of DM and CP content than *B. thonningii* leaves. ADF values of *B. thonningii* leaves were significantly ($P < 0.05$) higher than that of *D. cinerea* leaves. Significant difference ($P < 0.01$) were recorded for Ca, Mg, K, P and Fe for the two species. There were no difference ($P > 0.05$) in Na, Zn, Cu and Mn for *B. thonningii* leaves and *D. cinerea* leaves. In conclusion, *D. cinerea* and *B. thonningii* leaves showed that they contain high protein levels which can be used as a protein source.

Key words: chemical composition, mineral content, *Dichrostachys cinerea*, *Bauhinia thonningii*, browse trees

3.1. Introduction

Tree leaves form a natural part of the diet of many ruminant animals and have been used conventionally as sources of forages for livestock in Vhembe District and the rest of Africa. Browse constitutes an important fodder component for ruminants in tropical dry areas especially in the dry season when the available grazing is not of sufficient quality or quantity to meet the maintenance requirements of the animals (Aganga and Mosase, 2001). The objective of the current study was to determine the chemical composition of dry matter (DM) and crude protein (CP) of *Dichrostachys cinerea* and *Bauhinia thonningii* leaves. Browse forage maintain higher protein and mineral contents during growth than do grasses, which decline rapidly in quality as they progress to maturity (Aganga and Tshwenyane, 2003). The protein contents of the browse forages suggest that they have high potential for use as protein supplements in ruminant feeding. Breman and Kessler (1995) estimated the average concentration of crude protein (CP) in foliage of browse plants in the Sahelian and Sudanian areas of West Africa to be 150 g/kg DM, with variations between 100 and 206 g/kg DM, whereas Laudadio *et al.*, (2008) found an average CP content of 97 g/kg DM in fourteen plants collected in a pasture of Southern Tunisia, with variations between 41 and 165 g/kg DM for *Imperata cylindrica* L. and *Suaeda mollis* (Desf) Delile., respectively. In ruminants fed high quality fresh forage diets (25–35 g N/kg DM and 10–11 MJ of ME/kg DM) most proteins are rapidly solubilized and release between 56 and 65% of the N concentration in the rumen during mastication; consequently large losses of N occur (25 - 35%) as ammonia absorbed from the rumen occur (Min *et al.*, 2000). *D. cinerea* (g/kg) leaves contained 413 NDF, 374 ADF and 110 CP (Terfera *et al.*, 2008).

3.2. Materials and methods

3.2.1. Description of the Study Area

Dichrostachys cinerea and *Bauhinia thonningii* young leaves of were harvested in Shayandima, South Africa near Thohoyandou (23°0' 42.92" S, 30°25' 16.89" E) (Figure 1), 62 km east of Louis Trichardt, where the daily temperatures range between 10°C – 19°C in winter and >30°C in summer. The rainfall is seasonal and occurs during summer months from October to March ranging from 350 – 400 mm per annum (Kabanda *et al.*, 2015). The samples were analysed at the School of Agriculture, Animal Science Nutrition Laboratory, University of Venda, Thohoyandou, 85 km east of Louis Trichardt in the Limpopo Province of South Africa (22°58'32" S 30°26'45" E), at room temperature. The average and minimum temperature are 31°C and

18°C, respectively (Tardross *et al.*, 2005). The area is characterised by deep, well drained red clay soils (Soil Classification Working Group, 1991).

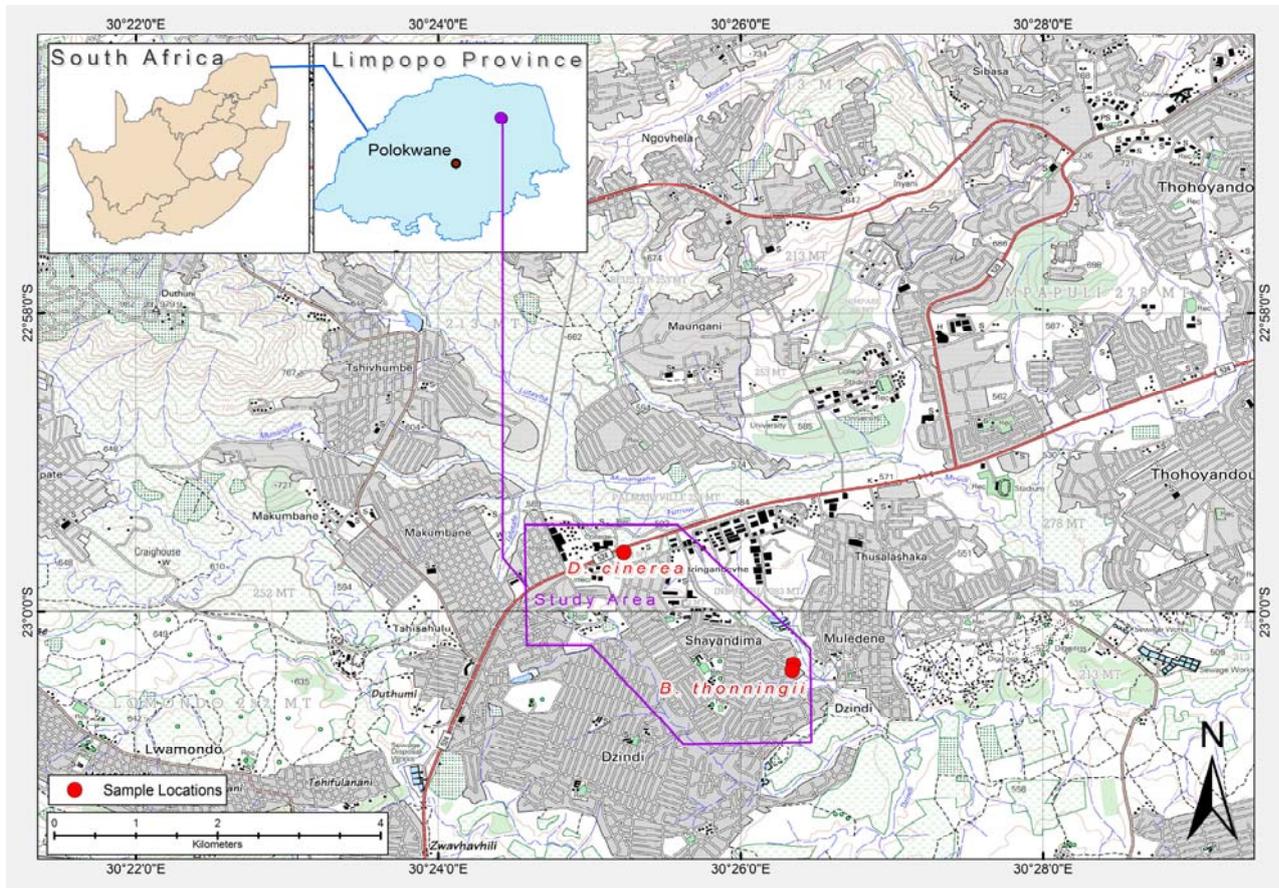


Figure 3.1: Map of Shayandima

3.2.2. Experimental design and sample collection

Five trees of each species growing in well drained red clay soils were marked with tree tags and the young leaves were harvested from those five selected trees at Shayandima as described above in 3.2.2. The leaves were harvested in late-wet season (February). Three new terminal shoots (leaf and petiole), made up of young leaf and twigs of less than 6 mm in diameter of *D. cinerea* and *B. thonningii* were harvested and placed in five separate bags (five bags for *D. cinerea* leaves and another five bags for *B. thonningii* leaves) and they were analysed separately. Each bag was treated for chemical analysis and replicated three times. The

experiment was arranged in completely randomized design (CRD) with two species serving as treatments.

3.2.3. Chemical analysis

Triplicate samples were milled to pass through a 1 mm sieve and analysed DM by drying in an oven at 60°C for 48 hours according to the method described by AOAC, (2000); method 976.05. Ash were determined by igniting samples overnight at 550°C (AOAC, 1990: method 923.03). Nitrogen was analysed using the Kjeldahl method (AOAC, 2000); method 984.13 and were converted to crude protein as $N \times 6.25$. NDF and ADF contents were determined using the method of Goering and van Soest, (1970).

3.2.4. Determination of mineral content

The forage legumes were ashed in a CNW Model SXL muffle furnace (manufactured by Trademark: Meditry, Origin: China) at the temperature of 600°C overnight. The ashed 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), magnesium (Mg), phosphorus (P), potassium (K), iron (Fe), sodium (Na), zinc (Zn), copper (Cu) and manganese (Mn). The Varian spectrophotometer instrument, model AA 20 equipped with a graphite tube atomizer, model GTA 110 (manufactured by Varian, Inc. in Palo Alto, California) with a programmable sample dispenser that diluted standards to the required concentration was used. Calibration of Ca, P and K elements was done using the Merck standards. The three macro elements were then determined by flame or hydride generation technique (AOAC, 2000).

3.2.5. Statistical analysis

Bauhinia thonningii and *D. cinerea* leaves data on nutrient content obtained from the quality of the leaves were subjected to analysis of variance (ANOVA) using the General Linear Model of MINITAB software version 17 (2014). Means were compared using the Tukey's procedure (Tukey, 1953)

The statistical model was:

Model: $Y_{ij} = \mu + S_i + \epsilon_{ij}$

Y_{ijk} = the observation: DM, Ash, CP, NDF, ADF, minerals, μ = overall mean to all observations, S_i = effect of i^{th} species, ϵ_{ijk} = random residual error

3.3. Results

The chemical composition of *B. thonningii* and *D. cinerea* leaves are presented in Table 3.1. Ash contents for both species were significant ($P < 0.01$), while DM, CP and ADF content differed significantly ($P < 0.05$). DM content of *D. cinerea* were higher compared to the DM content of *B. thonningii*. Ash content of *B. thonningii* was higher than that of *D. cinerea*. There was a significant difference ($P < 0.05$) in CP content of both browse species. The CP content of *D. cinerea* leaves were higher than that of *B. thonningii* leaves content significant. NDF for both species were significant ($P < 0.01$). NDF, which represent cellulose, hemicellulose and lignin, values were significantly higher ($P < 0.01$). ADF values of *B. thonningii* leaves were significantly ($P < 0.05$) higher than that of *D. cinerea* leaves.

The mineral content of *B. thonningii* and *D. cinerea* leaves are presented in Table 3.2. Significant difference ($P < 0.01$) were recorded for Ca, Mg, K, P and Fe. There was no difference ($P > 0.05$) on Na, Zn, Cu and Mn. *D. cinerea* leaves had high ($P < 0.01$) Ca and Mg content than *B. thonningii* leaves, while *B. thonningii* leaves showed high contents of K and P than *D. cinerea* leaves.

Table 3.1. Chemical composition (g/kg DM) of *B. thonningii* and *D. cinerea* leaves

Species	DM	Ash	CP	NDF	ADF
	g/kg	g/kg DM	g/kg DM	g/kg DM	g/kg DM
<i>B. thonningii</i>	238.3 ^a	69.8 ^a	211.5 ^a	641.3 ^a	552.1 ^a
<i>D. cinerea</i>	446.3 ^b	51.8 ^b	250.7 ^b	573.1 ^b	495.5 ^b
SEM	15.44	0.92	3.33	19.66	5.37
Significance	*	**	*	**	*

** $: P < 0.01$; * $: P < 0.05$; ^{ab} Column means with different superscripts differ significantly at $P < 0.05$; SEM: Standard error Mean; DM: dry matter; CP: crude protein; ADF: Acid detergent fibre; NDF: Neutral detergent fibre

Table 3.2. Macro-mineral (g/kg) and micro-mineral (mg/kg) composition of *D. cinerea* and *B. thonningii* leaves

Species	Macro-mineral (g/kg)					Micro-mineral (mg/kg)			
	Ca	Mg	K	Na	P	Zn	Cu	Mn	Fe
<i>B. thonningii</i>	6.4 ^a	1.7 ^a	9.8 ^a	1.7 ^a	2.3 ^a	21.0 ^a	11.2 ^a	55.0 ^a	477.2 ^a
<i>D. cinerea</i>	10.6 ^b	2.2 ^b	8.0 ^b	1.5 ^a	1.1 ^b	18.4 ^a	10.8 ^a	47.8 ^a	666.2 ^b
SEM	0.51	0.60	0.34	0.09	0.09	1.25	0.58	2.30	11.66
Significance	**	**	**	NS	**	NS	NS	NS	**

** $: P < 0.01$; NS: non-significant: $P > 0.05$; ^{ab} 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

3.4. Discussion

The chemical composition of *B. thonningii* and *D. cinerea* leaves clearly showed that there are species differences in DM, ash, CP, NDF and ADF content. The ash content was within the range values (32.5 – 95.8 g/kg DM) reported by Aganga *et al.* (2005). The ash content of *D. cinerea* leaves was lower than the ash content of *B. thonningii* leaves, which implies that the plant has a good or high organic components and a rather low inorganic or mineral constituent (Omoregie and Oluyemisi, 2010). The chemical composition of CP showed that there are species differences in the CP content of the legumes. The amount of protein a cow needs depends on the size, growth, milk production and stage of pregnancy (Moran, 2005) (Table 2.2.). In the present study, CP content in the leaves of the two species was more than the recommended range for early lactation (160 – 180 g/kg DM) in cows (Moran, 2005), but it's within the range of values reported by Roman (2003). In the present study, the high CP values (211.5 and 250.7 g/kg DM) in browse could correct deficient nitrogen basal roughage. According to APRU (1980), mature cattle require 78 g/kg DM CP for maintenance and 100 g/kg DM CP for growing beef cattle. Other authors reported high CP contents (103 – 336 g/kg DM) in browse (Abdulrazak *et al.*, 2000). The CP content of browse leaves harvested at lower and middle tree

trunk was 141 g/kg DM (Rubenza *et al.*, 2003). However, the variations with the present study could be due to proportion of foliage sampled for analysis as well as stage of maturity. For example, Topps (1997) reported low CP contents of 153 – 219 g/kg DM in old leaves and 210 – 319 g/kg DM in newly emerged leaves. The differences can be explained by genotype factors that control differential accumulation of nutrients in leaves as related to stage of maturity and even the effectiveness of rhizobial nitrogen fixation. However, the concentration of CP in this study are still high enough to be used as protein supplements to mature natural grasses, which are frequently deficient in protein (Minson, 1988). The CP content of *B. thonningii* leaves were comparable to the levels observed by Tijani (2012). The high protein content in *B. thonningii* and *D. cinerea* leaves may be associated with continuous supply of nitrogen from rhizobial fixation (Norton, 1982). The variations in the protein content of the browse forages may also be related to the different proportions and composition of leaf fractions of the plant. *D. cinerea* is leafy as compared to *B. thonningii*. Leafiness in pasture plants is commonly associated with forage quality because there is usually positive correlation between leaf percentage in each plant species, protein and mineral composition (Norton, 1982). NDF and ADF concentration was more than the recommended requirements for cow's diet for healthy rumen function (300g/kg DM NDF and 190 g/kg DM ADF).

Variations in the levels of calcium (Ca), magnesium (Mg), potassium (K) and phosphorus (P) in the present study could be partly explained by different forage species, species composition and variation in soil characteristics due to location of different species (Mirzaei, 2005). The study showed that *B. thonningii* and *D. cinerea* leaves contained low to moderate levels of most micro minerals. *Dichrostachys cinerea* leaves had low levels (47.8 mg/kg) of Mn than *B. thonningii* leaves (55.0 mg/kg). Rubenza *et al.* (2006) concluded that species can be used as protein and mineral supplements for ruminants in the tropics due to their high levels of CP, Ca, P, S, Mn and Co. However the low concentration of Cu and Zn could be limiting in ruminant feeding. The mineral content for *D. cinerea* leaves were similar to the results observed by Chibinga and Nambeye (2016). A variation on mineral content for *B. thonningii* leaves was observed with the results observed by Chibinga and Nambeye (2016). However, the variation could be due to type of soil and climatic or seasonal conditions (Underwood, 1981). Calcium is normally one of the primary limiting factors in the diets of sheep and goat and hence they need to be provided as a supplement. Up to 99% of the Ca and 80% of the P in the entire body are found in skeleton and teeth (McDonald *et al.*, 2002). The Ca content concentrations of 2 - 6 g/kg, with higher requirements for lactation have been variously recommended for cattle and sheep National

Research Council (National Research Council, 1978, 1984, 1985: ARC, 1980). In the present study, Ca and Mg concentration of both species were within the range observed by Nitrim (2016). Abdullah *et al.* (2013) recommended the range of 1.9 – 8.2 g/kg Ca and 1.2 – 4.8 g/kg P for all classes of ruminants. In the current study, the level for *D. cinerea* exceeds the recommended range while the amount of P for *D. cinerea* and *B. thonningii* leaves are within the recommended range. Excessive K 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). 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). 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). The observed levels of Na in this study were comparable to the results observed by Morris (1980). The levels of P observed in this study were comparable to the levels referred in literature Nitrim, 2016, NRC, 1989, and NRC, 1996).

Zinc (Zn) concentrations were comparable to the results observed by NRC (2016). Copper (Cu) concentrations were comparable to the results observed by Nutrinim (2016). Manganese (Mn) concentration in the present study 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. Similar levels of Mn concentrations have been reported (Jedara *et al.*, 1987) and Prabowo, 1990). The iron (Fe) concentration of *D. cinerea* leaves found in the study is an agreement with the higher forage Fe value of 650 mg/kg reported by Khan (2003).

3.5. Conclusion

Dichrostachys cinerea leaves contained high CP content than *B. thonningii* leaves. The high protein level in *B. thonningii* and *D. cinerea* leaves implies that can be used as protein supplement in ruminant's diet. The mineral most widely present in inadequate amount in both species is sodium (Na) for dairy cattle.

CHAPTER 4: DETERMINATION OF RUMEN DEGRADABILITY OF *BAUHINIA THONNINGII* AND *DICHROSTACHYS CINEREA* LEAVES

Abstract

Crude protein (CP) degradation in the rumen is a very complex process and its quantification is an ongoing challenge to ruminant nutritionists. Rumen undegraded protein (RUP) for an individual feed is required by most feed evaluation systems in the calculation of metabolizable protein. The objectives the current study was to determine the rumen degradability of dry matter (DM) and CP of *Dichrostachys cinerea* and *Bauhinia thonningii* leaves. The browse tree leaves were harvested in the wild in Shayandima, Limpopo province. The leaves were collected, oven-dried, milled to pass through 1.0 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 (41 µm) and incubated in duplicates for 0, 4, 8, 16, 24, 48, 72, 96 and 120 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 outflow rate. DM and CP disappearance decreased ($P < 0.01$) as the incubation period increased. Low ($P < 0.01$) soluble CP components has been degraded in fraction ‘a’ for both species. For slowly degradable CP fraction ‘b’, the values for *B. thonningii* (85.7%) and *D. cinerea* (88.7%) were high ($P < 0.01$), suggesting a source of rumen degradable protein. Effective degradability increased ($P < 0.01$) with an increase in outflow rates. The results showed low DM and CP degradability which concludes that the leaves were not utilized by the rumen microbes.

Key words: dry matter disappearance, crude protein disappearance, *Dichrostachys cinerea*, *Bauhinia thonningii*, browse trees

4.1. Introduction

Browse leaves are important sources of high quality feed for grazing ruminants and as supplements to improve the productivity of herbivores fed on low quality feeds (Aganga and Tshwenyane, 2003). During dry season, the natural pastures and crop residues available for animals after crop harvest are usually fibrous and devoid of most essential nutrients including protein, energy and minerals which are required for increased microbial fermentation and improved performance of the host animal. During the dry season in Vhembe, there is usually a substantial amount of green fodder from planted and naturally occurring trees which can be used as a source of feed for smallholder farm animals.

Digestion in the rumen involves a sequential attack by ruminal microorganisms on feed (Cheng *et al.*, 1991). *In situ* rumen degradability determines the disappearance of feeds incubated in a porous bag within the rumen to estimate both the extent and rate of degradation. The procedure provides a basis of formulating rations to meet protein requirements of livestock (Ørskov, 2000). The *In situ* technique is particularly effective in this role and will have a valuable part in unravelling the most important feeding constraints in tropical feeding systems (Ørskov *et al.*, 1980).

The method is considered to be a reference method to estimate degradation parameters such as soluble “a”, insoluble but degradable and undegradable fractions “b”, outflow rate “c”, potential “a + b” and effective degradability (ED) by applying the equation, $P = a + b(1 - e^{-ct})$ (Ørskov and McDonald, 1979). These parameters are used by feeding evaluation models to estimate the nutritive value, nutrient supply, predict feed intake and growth rate (Ørskov *et al.*, 1988; Hackmann *et al.*, 2010). For example, high rates of degradation have been implied to result in high voluntary intake and thus higher performance (Sun *et al.*, 2012). The effective degradability of a given feed depends on the production level of the animal concerned, and thus on the outflow, as well as on the specific degradability pattern for a particular feed (Griffiths, 2004).

The degradability of plant in the rumen is related to the proportion and lignification of cell wall. Tree forages with a low neutral detergent fibre (NDF) content (20 – 35%) usually have higher digestibility while species containing lignin often have low digestibility (Tjelele, 2006). The low degradability of forages with high NDF and tannins is due to reduced penetrative ability to rumen microbes through lignified plant cell walls and precipitations effects of tannins to microbes and enzymes (Makkar *et al.*, 2003; Baloyi *et al.*, 2008). Antinutritional factors in

browse species cause precipitation of microbes and enzymes which are supposed to cause breakdown of the molecules (Makkar, 2003). Forages with low fibre to have high effective dry matter degradability compared to those with high fibre content (Limas-lamas and Combs, 1990 and Njidda *et al.*, 2012). This decrease in DM degradability can be attributed to the effects of condensed tannins on accessible N, which can decrease ammonia concentrations and microbial growth in the rumen (Salem *et al.*, 2007). The minimum level of crude protein (CP) required for microbial activities in the rumen is 7% (Njidda, 2011). There was a significant variation in DM and CP degradation parameters of multipurpose trees (Melaku *et al.*, 2003, Anele *et al.*, 2009 and Balgees *et al.*, 2013).

The objectives of the study were to determine the dry matter and protein degradability of *B. thonningii* and *D. cinerea* leaves.

4.2. Materials and method

4.2.1. Experimental site

The trial was carried out at the University of Venda experimental farm and Animal Nutrition Laboratory (22°56'60" S, 30°28'60" E), Limpopo, South Africa, as described in chapter 3.

4.2.2. Sample preparation

B. thonningii and *D. cinerea* leaves were harvested in Shayandima (Figure 1) and taken to the University of Venda, Animal Nutrition laboratory. The leaves were weighed and oven dried at 60°C for 48 hours according to the method described by AOAC, (2000); method 976.05. The leaves were ground to pass through 3 mm sieve. Approximately 5 g of samples were weighed and inserted in nylon bags.

4.2.3. Ethical consideration

Before the research trials with animals start, approval by the Ethical Committee of the University of Venda were sought and granted, Project no: SARDF/17ANS/01.

4.2.4. Animals and management

Three Bonsmara steers fitted with rumen cannulae of 8 cm internal diameter (ANKOM-flexible cattle purchased from Bar Diamond Inc., USA) were used to determine the profiles of dry matter (DM) and nitrogen (N) degradability of *B. thonningii* and *D. cinerea*. The animals were placed in one pen with clean water available during the whole experiment. The animals were fed with commercial complete cattle finisher (Table 4.1.) diet *ad libitum*.

Table 4.1. The chemical composition of commercial complete cattle finisher diet that was used

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)

4.2.5. Experimental design for ruminal degradability

For each 2 species, three new terminal shoots (leaf and petiole) of *D. cinerea* and *B. thonningii* were harvested in the same area and placed in ten marked bags (five bags for *B. thonningii* leaves and five bags for *D. cinerea* leaves). Each bag was treated and replicated three times. The experiment was arranged in completely randomized design (CRD) with two species serving as treatments.

4.2.6. Rumen incubation procedure

The Nylon bag technique (Ørskov and McDonald, 1979) were used. Well-labelled nylon bags (6 × 12 cm) with a pore size of 41 µm were filled with approximately 5 g of the browse samples. The bags were attached using plastic bands flexible vinyl plastic tubes, approximately 40 cm long to 6 mm outer diameter, and were then inserted into the rumen of the cannulated Bonsmara steers for incubations of 0, 4, 8, 16, 24, 72, 96 and 120 hours. The sample bags were duplicated per animal and incubation period in the rumen giving a total of 420 samples. The bags for one incubation hour (about 20 bags per steer) were incubated for the first time followed by last 6 incubation hours (about 20 bags per steer). All the bags were placed simultaneously in the rumen before the morning feeding. After each time interval elapse, the bags were removed and washed in a running tap water until the water becomes clear. The bags were dried using a forced-air oven at 60°C for 12 hours. The final residues in all bags were composited by the browse leaves treatment, incubation hour and steers and subsequently ground through a 1 mm sieve and analysed in duplicates. The bags were allowed to cool down and weighed to determine the DM disappearance. The residues were then analysed for nitrogen Kjeldahl method (AOAC, 2000); method 984.13.

The bags were inserted in the rumen at 06:00 h before the morning feeding time. Immediately at the end of each incubation time, the bags were removed from the rumen, immediately washed gently under low running tap water while rubbing gently between thumb and finger, till the water was clear and rinsed with deionized water. The zero hour (control) bags were washed without incubation in the rumen

4.2.7. Mathematical calculations

The nutrient degradation was calculated by the difference between the amount in control sample and degraded residues. The degradability of DM and protein with time for each sample was described using the mathematical model of Ørskov and McDonald (1979):

$$P = a + b (1 - e^{-ct})$$

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 (h^{-1})

Potential degradability (PD) were estimated as $(a + b)$, and the effective degradability (ED) where be calculated using the rumen fractional outflow rate (k) of 0.02 and 0.05 per hour according to Ørskov and McDonald (1979).

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

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 0.002 and 0.05

The degradation constants (a , b and c) were estimated with Neway Excel version 6 (The Rowett Research Institute, Aberdeen, UK).

4.2.8. Statistical analysis

Analysis of variance (ANOVA) on *B. thonningii* and *D. cinerea* leaves degradability data was performed at $P < 0.01$ and $P < 0.05$ according to Steel and Torrie (1980) using GLM procedures of Minitab Statistical package version 17 (Minitab, 2014). Significant difference between the means were compared using the Tukey's procedure. (Tukey, 1953). The Statistical model for ruminal degradability of *B. thonningii* and *D. cinerea* leaves was as follows:

$$Y_{ijk} = \mu + S_i + A_j + \epsilon_{ijk}$$

Where:

Y_{ijk} = the observation: ruminal degradability of DM and CP, ruminal kinetics, μ = overall mean to all observations, S_i = effect of i^{th} species, A_j = fixed effect of animal $i = 1, 2$ or 3 , ε_{ijk} = random residual error

4.2. Results

4.2.1. Degradability constants and Effective degradability

Mean degradability parameter values obtained by fitting the model of Ørskov and McDonald (1979), defining the kinetics of DM degradation and effective degradability at three rumen fractional outflow rates, are presented in Table 4.2. There was no difference ($P > 0.05$) in soluble fraction 'a' and 'b' of *B. thonningii* and *D. cinerea* leaves. A significant difference ($P < 0.01$) was observed between the two browse species. Effective degradability at three outflow rates varied significantly ($P < 0.01$). However, effective degradability (%) decreased when the rate of passage was increased from 2% to 8%.

Table 4.2. Degradability constants and calculated effective degradability at three passage rates for dry matter and crude protein disappearance of *B. thonningii* and *D. cinerea* leaves

		<u>Degradability constants</u>			<u>ED (%) at three outflow rates</u>		
		<i>a</i>	<i>b</i>	<i>c</i>	<i>k</i> = 0.02	<i>k</i> = 0.05	<i>k</i> = 0.08
DM	<i>B. thonningii</i>	12.1 ^a	87.9 ^a	0.005 ^a	37.7 ^a	21.7 ^a	17.0 ^a
	SEM	1.92	91.73	0.0008	0.033	0.030	0.449
	<i>D. cinerea</i>	8.2 ^a	91.7 ^a	0.005 ^b	33.0 ^b	18.6 ^b	15.2 ^b
	SEM	1.95	1.97	0.0008	0.03	0.03	0.46
	Significance	NS	NS	**	**	**	**
CP	<i>B. thonningii</i>	14.3 ^a	85.7 ^a	0.0015 ^a	34.6 ^a	20.0 ^a	18.5 ^a
	<i>D. cinerea</i>	11.3 ^b	88.7 ^b	0.0018 ^b	32.6 ^b	19.4 ^b	16.0 ^b
	SEM	0.05	0.05	0.000031	0.07	0.04	0.11
	Significance	**	**	**	**	**	**

***P* < 0.01; NS: *P* > 0.05. ^{ab} within a section in column, means with different superscripts are significantly different (*P* < 0.05). (SEM) Standard error Mean; (NS) not significant; DM: Dry matter; ED: Effective degradability

In situ DM disappearance (%) of *B. thonningii* and *D. cinerea* leaves are illustrated in Figure 4.1. The disappearance of DM increased (*P* < 0.01) as the period of incubation increase (0, 4, 8, 16, 24, 48, 72, 96 and 120 hours). *B. thonningii* leaves had high DM disappearance than *D. cinerea* leaves.

In situ CP disappearance (%) of *B. thonningii* and *D. cinerea* leaves are illustrated in Figure 4.2. The disappearance of CP increased (*P* < 0.01) as the period of incubation increase (4, 8, 16,

24, 48, 72, 96 and 120 hours). *B. thonningii* leaves had high CP disappearance than *D. cinerea* leaves.

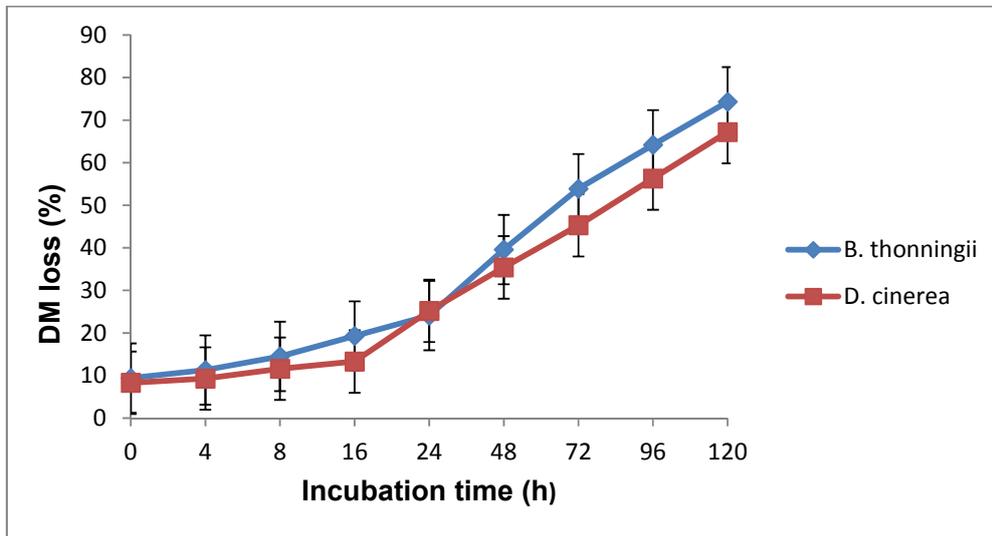


Figure 4.2: *In situ* dry matter disappearance (%) of *B. thonningii* and *D. cinerea* leaves

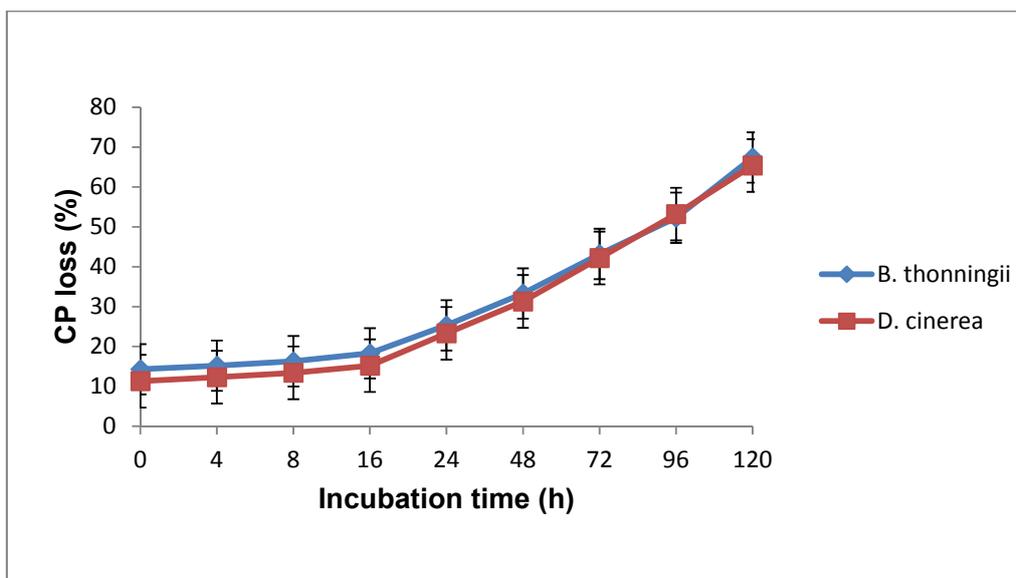


Figure 4.2: *In situ* crude protein disappearance (%) of *B. thonningii* and *D. cinerea* leaves

4.3. Discussion

4.3.1. *In situ* dry matter and crude protein degradability

In situ DM disappearance (%) of *B. thonningii* and *D. cinerea* leaves are illustrated in figure 4.1. Dry matter disappearance increased ($P < 0.01$) significantly over time. As the period of incubation increases, dry matter disappearance of the sample increases due to the increase in ruminal microbial composition which was penetrating inside the nylon bag which speed up the degradation of the sample, in the process the sample mass decreases due to more exposition to microbes. The disappearance of dry matter from the browse leaves in this study was observed to be moderate and well above 40% of their reported potential degradability (PD) values after 48hrs incubation. According to Ehargava and Ørskov (1987) high degradability values after 48hrs of incubation imply high digestibility since degradability values at this time are regarded as being equivalent to digestibility. Mon Seng *et al.*, (2001) reported that protozoal and bacterial populations in the rumen tended to increase with the increasing level of cassava leaf meal, especially the protozoa population, this is the reason why the degradation process at early hours rapidly increasing. The findings of this investigation showed DM disappearance values for 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 soluble DM contents indicate the potential to be good sources of more nutrients for microbial growth. Djouvinov and Todorov (1994) and Masama *et al.* (1997) reported a strong positive relationship between DM intake and microbial growth.

Browse species contain high levels of condensed tannins (Terrill *et al.*, 2010). Low crude protein degradability in tropical forage legumes in the presence of tannins has been reported by numerous authors (Ahn *et al.*, 1989; Kumar and Vaithiyathan, 1990). Tannins 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 proteins 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.*, 1994b). Condensed tannins have been shown to lower soluble 'a' protein and ammonia-N levels in ruminal fluid and promote greater nitrogen retention by reducing extra excretion and/or by increasing urea recycling to the rumen (McMahon *et al.*, 1999). Lower CP degradability in *B. thonningii* and *D. cinerea* leaves could also be due to high fibre content attributed in the leaves (Table 3.1) because fibre acts as a mechanical barrier

inhibiting microbial action (Van Soest, 1994), thus, rendering nutrient compounds unavailable during digestion (McDonald *et al.*, 2002). The difference with literature data and present study results could be a reflection of variations in plant growing conditions, season of the year, plant species, soil type, plant parts and stage of maturity (Norton and Waterfall, 2000; Aufrere *et al.*, 2003; Kasuya *et al.*, 2008) and anti-nutritional factors such as condensed tannins.

4.3.2. Dry matter and crude protein degradability kinetics

The fraction 'a' represent that there is a component which is being degraded rapidly and/or a component which is soluble. There was no difference in soluble fraction 'a' of DM. No difference was observed in fraction 'b' of DM. Effective degradability (ED) of DM decreased with an increase in outflow rate. Mupangwa *et al.* (1997) observed ED and DM to decrease as the outflow rate increased. *B. thonningii* and *D. cinerea* leaves showed high potential ($a + b$) degradability, indicating that they need to stay in the rumen for a longer time (Molina-Acaide *et al.*, 1996). Effective degradability (ED) of DM calculated at 2, 5 and 8% outflow rates from the rumen showed that *B. thonningii* had higher values than that of *D. cinerea* leaves.

Effective CP degradability was observed as was also observed with DM, decreased with increased in outflow rates. Similar observations relating to foregoing have been reported for dry matter and protein in legume forages (Mgheni *et al.*, 1996). Low soluble CP components has been degraded in fraction 'a' for both species. For slowly degradable fraction 'b', the values for *B. thonningii* (85.7%) and *D. cinerea* (88.7%) were high, suggesting a source of rumen degradable protein. However, Stock *et al.* (1984) reported that the average ruminal CP degradability of feed is 60 to 80 %. These differences in degradation may be associated to the structural and nonstructural protein and carbohydrate fractions (Belachew *et al.*, 2013). 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). 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 (Benjamin *et al.*, 1995) or maturity (Kamalak, 2006; Gusha *et al.*, 2013). The effective nitrogen degradability at passage rate 0.02 is within the range 32 and 80 per cent reported by Mgheni *et al.*, (1993) for some tropical legumes (*Desmodium intortum*, *D. uncinatum*, *Neanotonia wihitii*, *Pueraria phaseoloides* and *Leucaena leucocephala*). This high 'c' value indicates higher nutritive value of the browse forages and providing a high intestinal availability of undegraded

dietary nitrogen (UDN). The ED of DM and N in the *B. thonningii* and *D. cinerea* leaves calculated at an outflow rate of 2% per hour 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 could be providing valuable UDN to the intestine, with *D. cinerea* leaves providing more valuable UDN than *B. thonningii* leaves.

4.4. Conclusion

Significant variation in *in situ* degradability of *B. thonningii* and *D. cinerea* leaves was reported among two different browse species. The results observed showed low DM and CP degradability of *B. thonningii* leaves and *D. cinerea* leaves in the rumen which can be attributed to anti-nutritional factors in browse forages. Consequently, *D. cinerea* leaves showed a high potential supplementary protein source like high-quality leguminous forages compared to *B. thonningii* leaves.

CHAPTER 5: DETERMINATION OF *IN VITRO* DIGESTIBILITY OF *BAUHINIA THONNINGII* AND *DICHROSTACHYS CINEREA* LEAVES

Abstract

Metabolize protein available for absorption in the small intestine is dependent on the flow and digestibility of microbial crude protein and dietary ruminal undegradable protein. The objectives of the current study were to determine the *in vitro* digestibility of dry matter (DM) and crude protein (CP) of *Dichrostachys cinerea* and *Bauhinia thonningii* leaves. The browse tree leaves were harvested in the wild in Shayandima, Limpopo province. The leaves were collected, and transported to the Animal Science Nutrition Laboratory, at the University of Venda. The leaves were oven-dried, milled to pass through 3.0 mm sieve. Approximately 5 g of samples milled through a 3 mm sieve were placed in nylon bags (41 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). 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. The *in vitro* DM and CP degradability of rumen undegradable residue collected after 24 and 48 hour incubation was determined by sequential digestion in pepsin (abomasal) and pancreatin (small intestine) solutions. High dry matter degradability (DMD) and low *in vitro* dry matter digestibility (IVDMD) at 24 and 48 hours of incubation were observed. The CP digestibility of *B. thonningii* and *D. cinerea* leaves was increased ($P < 0.01$) from 24 and 48 hours of incubation. In conclusion, the results showed low CP digestibility of leaves, therefore leaving some for utilization by ruminants.

Key words: dry matter, crude protein, degradability, *in vitro* digestibility, *Dichrostachys cinerea*, *Bauhinia thonningii*, Pepsin-HCL solution, browse trees

5.1. Introduction

Forage evaluation implies the discretion of feedstuffs with respect to their capacity to sustain diverse kinds and levels (France *et al.*, 2000; Juarez *et al.*, 2004). Numerous previous studies focused on the nutritive value, degradability, voluntary intake and effects of anti-nutritive factors of browse legumes forages (Andualem *et al.*, 2016; Anele *et al.*, 2009; Balgees *et al.*, 2013; Melaku *et al.*, 2010; Njidda, 2011). However, few studies focused on post-ruminal digestibility, which makes it essential to determine the need for supplementation browse forages.

Digestibility is the measure of the gross availability of nutrients usually expressed in terms of dry or organic matter disappearance (Singh *et al.*, 2010). *In vitro* digestion techniques using rumen fluid as inoculum (Tilley and Terry, 1963) have proved useful in assessing the relative digestibility of many feedstuffs. (Minson, 1990). The main chemical component of feeds that determines the rate of digestion is neutral detergent fibre (NDF), which is a measure of cell wall content. Legumes contains less cell wall and are consumed in quantities about 20% greater than grasses (Forbes, 1986). Lignin and its cross-linkage to hemicellulose, polysaccharides and proteins could also depress digestibility (Van Soest, 1994.) Condensed tannins are present in fibres which bound to cell wall and protein, and appear to be responsible for decreasing digestibility (Reed *et al.*, 1990). Protein content and digestibility of dry matter have been emphasized as the main determinants of forage quality (Perez-Corena *et al.*, 1998).

In vitro digestibility is considered as the second step of feed evaluation (Mahmoud *et al.*, 2017). The crude protein (CP), fiber contents and dry matter (DM) degradability values are used as indicator to use as feed supplements for ruminant (Andualem *et al.*, 2016).

The objectives of the study were to determine the post-ruminal digestibility of dry matter and crude protein of *B. thonningii* and *D. cinerea* leaves

5.2. Materials and method

5.2.1. Experimental site

The trial was carried out at the University of Venda experimental farm and Animal Nutrition Lab (22°56'60" S, 30°28'60" E), Limpopo, South Africa as described in Chapter 3. The use of animals as well as protocols and procedures in this study were approved by the Ethical and Higher Degree Committees of The University of Venda as described in Chapter 3.

5.2.2. Bags specification and sample preparation

Samples used in the current experiment were prepared in Chapter 4.

5.2.3. Ethical consideration

The study was approved by the Ethics Committee of the University of Venda as described in Chapter 4, subsection 4.2.2.

5.2.4. Experimental design for post-ruminal digestibility

B. thonningii and *D. cinerea* leaves were used in the study. The two species were serving as treatment with three replications in each treatment. The experiment was arranged in a completely randomized design (CRD).

5.2.5. Determination of post-ruminal digestion of *B. thonningii* and *D. cinerea* leaves

a. Pepsin-HCL procedure

Approximately 5 g of ground leaves of *B. thonningii* and *D. cinerea* were weighed and put into a well-labelled nylon bags (6 × 12 cm, pore size of 41 µm). The nylon bags were attached using elastic bands, to flexible vinyl plastic tubes, approximately 40 cm long and of 6 mm outer diameter. The flexible vinyl plastic tubes were tied with 10 cm ropes and then secured to a rubber stopper. Triplicate bags per species treatment per incubation time per animal were inserted into the rumen and withdrawn at 24 and 48 incubation hours. The bags were inserted in the rumen at 06: 00 before the morning feeding time. After each incubation time, the bags were removed from the rumen, washed in a running tap water without squeezing, till runoff was clear, then finally washed with deionized water and dried at 60°C for 48 hours. Rumen undegraded residues (RUR) were removed from the bags manually. The RURs were composited by species treatment, incubation hours and the steers, and subsequently ground to pass through a 1 mm sieve.

b. Pepsin + pancreatic digestion procedure

For Pepsin + pancreatic digestion trial, a total of 3 bags containing 1 g of RURs of each *B. thonningii* and *D. cinerea* samples were introduced into incubation bottle which contained 2 l of a 0.1 N HCl solution adjusted to pH 1.9 with 1 g l⁻¹ of pepsin (P-700; Sigma), and were incubated for 1 hour with constant horizontal movement at 39°C. After incubation, the bags were rinsed with tap water till runoff was clear, then finally rinsed with deionized water and introduced into the incubation bottle containing 2 l of pancreatic 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 incubation, the bags were rinsed with tap, water until the run off was clear, then finally rinsed with deionized water and dried at 60°C for 48 hours. The final residues in all bags were composited by species treatment, incubation hours and steers and subsequently ground through a 1 mm sieve. The residues were analysed for DM and CP contents using the following methods:

RURs and *in vitro* undegradable residues were dried in a forced air oven at 60°C for 48 h (AOAC, 2000; method 976.05) 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 N content using the Kjeldahl procedure (AOAC, 2000; method 984.13) and N was converted to CP as N x 6.25.

5.2.6. Statistical analysis

Analysis of variance (ANOVA) on *B. thonningii* and *D. cinerea* leaves degradability data was performed at $P < 0.01$ and $P < 0.05$ according to Steel and Torrie (1980) using GLM procedures of Minitab Statistical package version 17 (Minitab, 2014). Significant difference between the means were compared using the Tukey's procedure. (Tukey, 1953). The Statistical model for ruminal degradability of *B. thonningii* and *D. cinerea* leaves was as follows:

$$Y_{ij} = \mu + S_i + A_i + \epsilon_{ijk}$$

Where:

Y_{ijk} = the observation: ruminal degradability of DM and CP, ruminal kinetics, μ = overall mean to all observations, S_i = effect of i^{th} species, A_j = fixed effect of animal $i = 1, 2$ or 3 , ϵ_{ijk} = random residual error

5.3. Results

5.3.1. *In vitro* dry matter digestibility

The ruminal degradability and *in vitro* digestibility data of DM of *B. thonningii* and *D. cinerea* leaves from the nylon bags are summarized in Table 5.1. DM disappearance increased as the period of incubation increased. There was a different ($P < 0.01$) in DM disappearance between the two selected browse species. *B. thonningii* leaves had higher DM disappearance and higher DM digestibility than *D. cinerea* leaves after 24 and 48 h of rumen incubation. DM digestibility of both browse foliage increased after 24 h of rumen incubation. *B. thonningii* leaves has been digested more than *D. cinerea* leaves at 48 h.

5.3.2. *In vitro* crude protein digestibility

The ruminal and *in vitro* digestibility of CP of *B. thonningii* and *D. cinerea* leaves from the nylon bags is summarized in Table 5.2. CP disappearance among the two selected browse foliage increased ($P < 0.01$) as the ruminal incubation period increase. *Bauhinia thonningii* leaves had higher CP disappearance but low CP digestibility than *D. cinerea* leaves after 24 h of rumen incubation, while *D. cinerea* had high CP disappearance than *B. thonningii* leaves at 48 h of incubation.

Table 5.1. Dry matter disappearance (%) after 24 and 48 hour incubation in the rumen and subsequent *in vitro* digestibility of *B. thonningii* and *D. cinerea* leaves

Species	DMD ₂₄	DMD ₄₈	IVDMD ₂₄	IVDMD ₄₈
<i>B. thonningii</i>	24.1 ^a	39.6 ^a	64.3 ^a	75.1 ^a
<i>D. cinerea</i>	25.2 ^b	35.4 ^b	61.2 ^b	70.5 ^b
SEM	0.07	0.07	0.06	0.13
Significance	**	**	**	**

***: P* < 0.01, DMD₂₄: Dry matter degradability at 24 hours incubation, DMD₄₈: Dry matter degradability at 48 hours incubation, IVDMD₂₄: *In vitro* dry matter digestibility after 24 hours of rumen incubation and IVDMD₄₈: *In vitro* dry matter digestibility after 48 hours of rumen incubation, ^{ab} Column means with different superscripts differ significantly at *P* < 0.05, SEM: standard error mean

Table 5.2. Crude protein disappearance (%) after 24 and 48 hour incubation in the rumen and subsequent *in vitro* digestibility of *B. thonningii* and *D. cinerea* leaves

Species	CPD ₂₄	CPD ₄₈	IVCPD ₂₄	IVCPD ₄₈
<i>B. thonningii</i>	25.3 ^a	33.3 ^a	17.1 ^a	22.4 ^a
<i>D. cinerea</i>	23.3 ^b	31.3 ^b	14.1 ^b	24.2 ^b
SEM	0.05	0.05	0.38	0.44
Significance	**	**	**	**

***: P* < 0.01; CPD₂₄: Crude protein degradability at 24 hours incubation; CPD₄₈: Crude protein degradability at 48 hours incubation; IVCPD₂₄: *In vitro* crude protein digestibility after 24 hours of rumen incubation and IVCPD₄₈: *In vitro* crude protein digestibility after 48 hours of rumen incubation, ^{ab} Column means with different superscripts differ significantly at *P* < 0.05, SEM: standard error mean

5.2. Discussion

5.4.1. *In vitro* dry matter digestibility

Legumes have generally faster rates of cell wall and also greater potentially undegradable fractions than grasses, due to higher lignin contents (Lopez et al., 1991). Variations in the

results among two species can be attributed to several factors, such as processing of feed, preparation of buffer solution, or handling of equipment (Tufarelli *et al.*, 2010).

The IVDMD values recorded in this study were generally higher. This could be due to values high fiber 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 (Calsamiglia and Stern, 1995). The digestibility values reported by Tesfay *et al.*, (2009) for browse are consistent with the findings of the present study. The IVDMD₄₈ values fall within the range (676.3 – 933.5 g/kg DM) reported by Aster *et al.*, (2012). The IVDMD values for *B. thonningii* and *D. cinerea* leaves observed in the present study was more than the critical threshold of 50% which is required for feeds to be considered as having acceptable digestibility (Owen and Jayasuriya, 1989). Digestibility values for browse legume classes were 67.8%, and this is in effect higher than the reported threshold levels (Buxton, 1996) and values previously reported by others (Bediye *et al.*, 1996) for browse species (55.1%).

5.4.2. In vitro crude protein digestibility

As the leaves matures, palatability and digestibility decline (Mpanza, 2015). In the current study, the leaves were harvested in late-summer wherein the leaves where matured. The results observed showed low CP degradability of *B. thonningii* and *D. cinerea* leaves. This can be attributed to forage quality which varies over the growing season and declines as the forage matures (Licitra *et al.*, 1997). However, browse from high-tannin species (*Acacia* or *Caliandra*) were digested better by goats adapted to consuming high-tannin feedstuffs (Tjakradidjaja *et al.*, 2000).

Blade *et al.*, (1993) reported that the stages of maturity affect both degradability and digestion and are associated with increase in the indigestible fraction of forage and an increase in the lignification of NDF. The results showed a high protein degradability in the rumen and low protein digestibility of *B. thonningii* and *D. cinerea* leaves, therefore available for utilization by ruminants.

5.4. Conclusion

The results showed that the rate of CP disappearance of *B. thonningii* and *D. cinerea* leaves increased but subsequently decline in post rumen digestion. High ruminal degradability can result in lower post rumen digestibility, therefore leaving an opportunity to improve the efficiency of nitrogen utilization by ruminants.

CHAPTER 6: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1. General discussion

The difference between the two species could be due to the effects of plant type, botanical factors and fertility, soil moisture, physiological and morphological characteristics, and may vary with annuals versus perennials, grasses versus legumes, etc. (Kilcher, 1981). Teferedegne (2000) reported that environmental differences influence the chemical composition of forages grown in different areas and harvested at the same age of maturity. The observed ash content of *D. cinerea* were comparable to the values obtained in the study of Aganga (2005). Raman (2003) reported that browses contain crude protein (CP) content ranging from 30 g/kg - 260 g/kg during dry season, the values observed in the present study were within this range. In the present study, the results of acid detergent fibre (ADF) content were variable but broadly comparable to the results of Aganga *et al.* (2005). The values observed for ADF were much higher among the species, this could be due the influence of genotype and environment (Mupangwa *et al.*, 1997). Little information has been found regarding the chemical composition of *B. thonningii* leaves.

Over 99% of total body calcium is found as calcium hydroxyapatite in bones and teeth, where it provides hard tissue with its strength (Shaker and Deftos, 2014). In the present study, calcium (Ca) content (6.4 g/kg) of *B. thonningii* could be enough for sheep while the Ca content (10.6 g/kg) for *D. cinerea* could be provided for cattle, the results were within the range of values reported by Nutrinim, 2016. Magnesium (Mg) concentration was adequate for lactating animals (NRC, 1986 & 1996), the observed results of Mg for both species were within the range values reported by Nutrinim, 2016, which is adequate of cattle. Potassium (K) concentration of *B. thonningii* and *D. cinerea* were above 8 g/kg recommended for grazing animals (Underwood, 1981), it may not meet the requirements (10g/kg) for high producing cows, under stress such as heat stress (Mirzaei, 2012). An adequate range of 1 – 4 g/kg of sodium (Na) has been recommended by Underwood (1981). To meet the need of highly productive animals, forage should contain more than 1.5 g/kg of Na (Mirzaei, 2012). In the present study, the results of both species were comparable with the results observed by Underwood (1981). P concentration of *B. thonningii* (2.3 g/kg) can be adequate for growing and lactating cows (NRC, 1981 and 1999), whereas P concentration of *D. cinerea* was can be adequate for sheep (Nutrinim, 2016).

It has been suggested that 30mg/kg to be a critical level of dietary zinc (Zn), although it has been recommended that concentrations of 12 – 20 mg/kg are adequate for growing ruminants (ARC, 1980) in the present study, Zn concentration of *D. cinerea* was 18.4 mg/kg and *B. thonningii* (21.0 mg/kg). Which can be recommended for sheep, growing cows and lactating cows. It is commonly suggested that the dietary requirement of copper (Cu) ranges from 8 - 14 mg/kg (Khan et al., 2006), the results observed in the present study for both species are within this range. High levels of Mn can retard the growth of livestock ((Mirzaei, 2012). Manganese (Mn) levels of *B. thonningii* and *D. cinerea* were above 40 mg/kg, the critical level and found to be sufficiently higher to meet the requirements of ruminants. Similar levels of forage Mn has been reported (Velssquez-Pereira, 1997 and Khan *et al.*, 2007). Variations in the iron (Fe) contents among the two species could be partly explained by forage species' differences and the influence of grazing land on the level of Fe in the soil. Both species had high levels of Fe and the variation in the contents of Fe with literature could also be related by variations in the content of Fe in the soil, and climatic conditions (Ramirez *et al.*, 2004 and Mirzaei, 2012). Limited information has been found regarding mineral content of *B. thonningii* and *D. cinerea* leaves.

However, microbial populations in the rumen of goats fed a diet supplemented with the same browse species may evolve to become resistant to secondary compounds, in particularly condensed tannins, thereby becoming superior at degrading feedstuffs rich in condensed tannins (Camacho *et al.*, 2010). Cross-linkages of lignin to hemicellulose, polysaccharides and proteins may also depress digestibility (Van Soest, 1994). In the current study, the lignin content of browse species was likely high, as suggested by the positive relationship between neutral detergent fibre (NDF) and ADF in the studies of Frutos *et al.*, (2002), and this could explain the lower of dry matter (DM) and NDF degradability of browse during the dry season.

The species studied had less than 50% effective degradability (ED) values. *B. thonningii* leaves had high effective degradability than *D. cinerea* leaves when calculated using the outflow rate of 2, 5 and 8%. According to Mupangwa (2003) variations on ED of dry matter in forages closely corresponds with the proportion of potentially degradable dry matter and level of NDF. However, Ørskov and McDonald (1979) showed that with higher outflow rates, less is degraded, in the present study, the reduction in degradability was much higher when the rate of passage was increases from 2% to 8%. The rapidly degradable CP fraction 'a' was generally low across the browse leaves studied. This is possibly an indication of high lignification in most of the leaves or may have, according to Adogba-Bessa and Owen (1995), resulted from accumulation of soluble

carbohydrates due to later stage of maturity. The insoluble but degradable CP fraction 'b' was observed to be high in *B. thonningii* and *D. cinerea* leaves. This observation may probably be due to cell wall content (Wilson, 1994). The rate of degradation 'c' is important in determining effective degradation 'a' well as rumen fill (McDonald *et al.*, 2002). The low DM and CP values of degradability in this study indicate a possible significant tannin-induced depression for *B. thonningii* and *D. cinerea* leaves.

6.2. Conclusion

This experiment 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 *B. thonningii* and *D. cinerea* leaves. The browse leaves showed good nutritional quality in terms of their ruminal disappearance of CP. The CP content of *B. thonningii* and *D. cinerea* leaves remained relatively high suggesting the possibility that leaves may be used as dry season fodder and protein supplement to low-quality diets.

6.3. Recommendations

It is recommended that *B. thonningii* and *D. cinerea* leaves can be used as form of potential feed resources mainly as protein supplements to ruminants fed on low quality basal forages especially during dry season. Therefore:

- i. There is a need to encourage farmers in communal areas wherein the livestock are adapted to browsing legume forage, to use *B. thonningii* and *D. cinerea* leaves as protein source in dry season.

Further research is necessary to study the following:

- i. Anti-nutritional factors in *B. thonningii* and *D. cinerea* leaves
- ii. Treatments to avoid negative effects of condensed tannins in ruminants still need to be done.
- iii. Treatments to protect dietary protein from ruminal degradation

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APPENDIX

Appendix 1: Analysis of variance for chemical composition (g/kg DM) for *B. thonningii* and *D. cinerea* leaves

Source	df	DM	Ash	CP	ADF	NDF
Species	1	108225*	2421.80**	11530.3*	23992.8**	34898*
Error	24	4769	306.80	3985.7	10370.5	139132

** $: P < 0.01$; * $: P < 0.05$. 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 *B. thonningii* and *D. cinerea* leaves

Source	df	Ca	Mg	K	Na	P	Zn	Cu	Mn
Species	1	42.436**	0.57600**	8.1000**	1.0000	28.224**	3.4810	0.400	129.60
Error	8	10.348	0.14000	4.7240	0.34400	2.096	0.3480	19.600	200.80

** $: P < 0.01$; * $: P < 0.05$. 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 *B. thonningii* and *D. cinerea* leaves

		Incubation time (h)								
		0	4	8	16	24	48	72	96	120
DM	<i>B. thonningii</i>	14.3 ^a	15.2 ^a	16.3 ^a	18.3 ^a	25.3 ^a	33.3 ^a	43.2 ^a	53.3 ^a	67.4 ^a
	<i>D. cinerea</i>	11.3 ^b	12.3 ^b	13.4 ^b	15.2 ^b	23.3 ^b	31.3 ^b	42.2 ^b	53.2 ^b	65.3 ^b
	SEM	0.05	0.04	0.04	0.04	0.05	0.05	0.07	0.04	0.04
	Significance	**	**	**	**	**	**	**	**	**
CP	<i>B. thonningii</i>	9.4	11.3	14.5	19.3	24.1	39.6	53.9	64.2	74.3
	<i>D. cinerea</i>	8.3	9.3	11.6	13.3	25.2	35.4	45.3	56.3	67.5
	SEM	0.06	0.05	0.06	0.05	0.06	0.07	0.07	0.05	0.05
	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 *B. thonningii* and *D. cinerea* leaves

		Degradability Constants			Effective degradability (%)		
Source	df	<i>a</i>	<i>B</i>	<i>c</i>	<i>K</i> =0.02	<i>K</i> =0.05	<i>K</i> =0.08
Species	1	220.1	212.5	0.0000022**	319.790**	138.767**	48.750**
Error	53	5852.3	5937.2	0.00000.90	1.687	1.460	320.717

** $P < 0.01$; 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 *B. thonningii* and *D. cinerea* leaves

Source	df	Degradability constants		Effective degradability (%)			
		A	B	c	K=0.002	K=0.05	K=0.08
Species	1	130.8	130.8	0.0000019	59.0	94.4	97.1
Error	58	4.8	4.8	0.0000017	9.4	2.9	19.2

** $: P < 0.01$; 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 *B. thonningii* and *D. cinerea* leaves

Source	df	DMD ₂₄	DMD ₄₈	IVDMD ₂₄	IVDMD ₄₈
Species	1	18.5393**	262.390**	1418.1**	3259.8**
Error	58	6.4246	8.387	67.4	303.7

** $: P < 0.01$; df: Degree of Freedom, DMD₂₄: Dry matter degradability at 24 hours incubation; DMD₄₈: Dry matter degradability at 48 hours incubation; IVDMD₂₄: *In vitro* dry matter digestibility after 24 hours of rumen incubation and IVDMD₄₈: *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 *B. thonningii* and *D. cinerea* leaves

Source	df	CPD ₂₄	CPD ₄₈	IVCPD ₂₄	IVCPD ₄₈
Species	1	59.5**	61.7**	135.4**	47.0**
Error	58	4.5	4.7	257.0	334.4

** $: P < 0.01$; df: Degree of Freedom, CPD₂₄: Crude protein degradability at 24 hours incubation; CPD₄₈: Crude protein degradability at 48 hours incubation; IVCPD₂₄: *In vitro* crude protein digestibility after 24 hours of rumen incubation and IVCPD₄₈: *In vitro* crude protein digestibility after 48 hours of rumen incubation.