NUTRIENT CONTENT AND THE EFFECTS OF FEEDING FOUR NAMIBIAN ENCROACHER BUSH SPECIES ON GROWTH, METHANE PRODUCTION AND CARCASS CHARACTERISTICS OF DAMARA SHEEP

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KATRINA LUGAMBO SHININGAVAMWE

200032674

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MAIN SUPERVISOR: Prof. Johnfisher Mupangwa (University of Namibia)
CO-SUPERVISOR: Dr. Emmanuel Lutaaya (University of Namibia)

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ABSTRACT

The aim of this study was to evaluate the nutritional and feeding value of four Namibian encroacher species on the intake, digestibility, growth performance and carcass characteristics of Damara sheep. In the first experiment, the nutritional quality of four encroacher bush species (*Senegalia mellifera*, *Dichrostachys cinerea*, *Terminalia sericea* and *Rhigozum trichotomum*) was evaluated. Leaves and twigs (< 20mm stem diameter) harvested during the late dry, early rainy and late rainy seasons were analysed for proximate content, minerals, amino acids, fatty acids and anti-nutrient composition. In the second experiment, *in vitro* dry matter digestibility, *in sacco* neutral detergent fibre digestibility and methane production on the same samples above were evaluated. In the third experiment, milled bush biomass was evaluated as alternative roughage source in total mixed rations fed to five castrated 13 month old Damara sheep, with an average initial body weight of 37.2 ± 2.4 kg at 40% inclusion rate in diets, with the main focus on the nutrient intake, *in vivo* dry matter digestibility and nitrogen retention. In the fourth and fifth experiments, the feed intake, growth performance, slaughter weights and carcass characteristics of Damara sheep lambs (15 males and 15 females) with an average initial weight of 16.7 ± 1.9 kg fed bush-based diets were evaluated. The results of the study indicated that most nutrients analysed were influenced ($P < 0.0001$) by season x species interaction. The crude protein (CP) contents was moderate (70 to 111 g/kg dry matter (DM)) except for *T. sericea* which was below 50 g/kg DM. The ash-free neutral detergent fibre (NDFom) and ash-free acid detergent fibre (ADFom) contents were high for all species (594 to 734 g/kg DM and 463 to 580g/kg DM, respectively), across all seasons. The concentration of acid detergent lignin of species ranged from 138 g/kg DM (*R. trichotomum*) to 223 g/kg DM (*D. cinerea*) but varied across seasons within species. A moderate proportion (50.9-56.7g/100g CP) of protein in *R. trichotomum* and *S. mellifera* was soluble, while the other species had a high proportion (> 70 g/100g CP) of their CP bound to ADF. The levels of condensed tannins (CT) was relatively low (< 55 g CT/kg DM) in all species and within the safe limits. All the bush species had low to moderate concentrations of minerals, while concentration of total amino acids and fatty acids was in the range of 39.4 to 77.7 g/kg DM and 1.17 to 2.84 g/kg DM, respectively. *In vitro* methane gas production of all four species was higher ($P < 0.001$) during the late dry season compared to the early rainy season (147.6 versus 92.0 mL/g 138 DM). The *in vitro* organic matter digestibility of the species decreased ($P < .001$) from late dry to early rainy season, except for *S. mellifera*. Increase of indigestible neutral detergent fiber ($P < .001$) was observed from late dry to early rainy season for other species, except for *S. mellifera* which decreased. Dry matter and CP intakes of the control diet was higher than the bush-based diets. Digestibility coefficients of ≥ 0.70 were obtained on bush-based diets for all nutrients except for NDFom and ADFom which ranged from 0.40 to 0.60. Positive nitrogen retention of 45-58% of N intake was also achieved across diets. The bush-based diets supported average daily gains of up to 156 g/day, feed conversion ratio of 7.6 to 9.6 kg feed/kg weight gain, carcass weights of 11.4 to 12 kg and their carcasses characteristics were similar ($P > 0.05$) to the control diet. In conclusion, milled bush species could be considered of intermediate nutritional quality, despite their high fibre fraction, high proportion of their CP bound to ADF and indigestible NDF. Milled bush can replace traditional roughage sources such as grass and lucerne hay at 40% inclusion rate in properly formulated and balanced ruminant diets without adverse effects on the dry matter intake, digestibility, growth performance and carcass characteristics of growing lambs. However, there is still a need for further research to improve the utilisation of these bush species.

DEDICATION

This work is dedicated to my children, Katrina Ndahekelekwa and Kiandre Tangi.

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ABBREVIATIONS	**DESCRIPTIONS**
ADF	Acid detergent fibre
ADFom	Ash free-Acid detergent fibre
ADG	Average daily gain
ADIP	Acid detergent insoluble protein
ADL	Acid detergent lignin
ADLom	Ash free-Acid detergent lignin
ADSP	Acid detergent soluble protein
ALA	Alpha linolenic acid
AMF	Arbuscular mycorrhizal fungi
ANF	Anti-nutritional factor
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AREC	Animal Research and Ethical Committee
BE	Bush equivalent
BECVOL	Biomass estimates from canopy volume
Ca	Calcium
CCD	Cold carcass dressing
CCW	Cold carcass weight
CEL	Carcass external length
CNCPS	Cornell net carbohydrate and protein system
CP	Crude protein

CRD	Complete randomized design
CT	Condense tannin
Cu	Copper
DM	Dry matter
DMI	Dry matter intake
dNDF	Digestible neutral detergent fibre
DVS	Directorate of Veterinary Services
EAA	Essential amino acids
EE	Ether extract
ERS	Early rainy season
EU	European Union
EU	Experimental unit
FA	Fatty acid
FAME	Fatty acid methyl esters
FCR	Feed conversion ratio
Fe	Iron
FLW	Final live weight
GCMS	Gas-liquid chromatography-mass spectrometry
GDP	Gross domestic product
GHG	Greenhouse gas
GLM	General linear model
GPS	Global positioning system
HCW	Hot carcass weight
HPC	High protein concentrate
HT	Hydrolysable tannin
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
iNDF	Indigestible neutral detergent fibre
JAP	John Alphons Pandeni Research Station
K	Potassium
KAL	Kalahari Research Station
Kg	Kilogram

LDS	Late dry season
LRS	Late rainy season
MAWF	Ministry of Agriculture Water and Forestry
MCA	Millennium Challenge Account
ME	Metabolisable energy
Mg	Magnesium
Mg	Milligram
Mn	Manganese
N	Nitrogen
Na	Sodium
NBT	Nylon bag technique
NDF	Neutral detergent fibre
NDFom	Ash free-neutral detergent fibre
NDSP	Neutral detergent soluble protein
NEAA	Non-essential amino acid
NPC	National Planning Commission
NPN	Non protein nitrogen
NRC	National Research Council
OM	Organic matter
OMD	Organic matter digestibility
P	Phosphorus
pdNDF	Potential digestible neutral detergent fibre
peNDF	Physically effective neutral detergent fibre
PVPP	Polyvinyl-polypyrolidone
SAN	Sandveld Research Station
SAS	Statistical analysis software
SEM	Standard error of means
SFA	Short-chain fatty acid
SolP	Soluble protein
SSMS	Small stock marketing scheme
SW	Slaughter weight

TFA	Total fatty acid
TP	Total phenol
TT	Total tannin
UPLC	Ultra-performance liquid chromatography
UREC	University of Namibia Research Ethic Committee
VFA	Volatile fatty acid
WW	Weaning weight
Zn	Zinc

CHAPTER 1

Introduction

1.1 Background of the study

In Namibia, livestock production is a major economic and livelihood activity, which contributes at least 75% to total agricultural output, with beef production being in the lead, followed by sheep and goats production (MAWF, 2012). Namibia has a comparative advantage in exporting sheep and is one of the few African countries that meet the strict import regulations of the EU, which demonstrated that sheep farming is profitable and competitive (Oosthuizen and Laubscher, 2019). While Namibia has several breeds of sheep for commercial trade, the Dorper sheep is the prime breed, which makes up approximately 45% of the sheep herd in the country (Oosthuizen and Laubscher, 2019). However, Damara, Van Rooy and Swakara sheep are also important breeds due to their adaptability to a wide range of climatic conditions (Tshabalala *et al*., 2003) and can thrive well in dry areas where feed resources are limited (Erasmus, 2000).

According to Oosthuizen and Laubscher (2019), the livestock industry contributed about 2.6% to the country's gross domestic product (GDP) and 25% of the foreign exchange earnings in 2018. The livestock industry is export-orientated with about 80% of annual production being exported mainly to South Africa and the rest to the European Union (EU) markets (Meat Board Namibia, 2020). However, the industry faces challenges including prolonged droughts, increased production costs, bush encroachment, low and erratic rainfall, variable climatic conditions, disease outbreaks and policy imperatives influencing livestock production, marketing and trade (Oosthuizen and Laubscher, 2019).

Amongst many threats to the industry, bush encroachment and prolonged drought spells pose a major concern (Oosthuizen and Laubscher, 2019). It is estimated that up to 45 million hectares of rangelands are affected by bush encroachment, resulting in reduced carrying capacity (Shikangalah and Mapani, 2020) and posing a further threat to livestock productivity (Archer *et al*., 2017). In addition, forage availability is worsened by recurrent droughts often associated with animals loosing condition, reduced reproduction and increased mortality rates (Sweat and Burke, 2000; Reid *et al*., 2007). There is evidence that with the arid nature of the country, coupled with climatic variability, livestock production has been continuously decreasing over the years (Spear *et al*., 2018). Statistics by Oosthuizen and Laubscher (2019) indicated that cattle numbers in Namibia declined from 2.9 million in 2012 to 2.7 million in 2017, while the total number of sheep declined from 2.7 million in 2012 to 1.7 million in 2016, then increased to 2.1 million in 2017. This further causes economic loss every year to the livestock industry, which is estimated to be more than one billion Namibia dollars (National Rangeland Management Policy, 2012).

Despite the bush encroachment challenge, the encroacher woody browse plants still remain potential animal feeds. They provide considerable amount of biomass from leaves and small twigs as well as pods and maintain their nutrients into the dry season when other feed resources are depleted (Moleele, 1998; Chinembiri, 1999) and are utilized in dry season feeding (Katjiua and Ward, 2007; Sweet and Burke, 2002; Seigmund-Schultze et al., 2012). Hence, the rehabilitation and management of encroached lands should not ignore the role of browse plants in the diets of livestock, even in good rainfall years. Interventions should aim at converting massive encroacher bush biomass resources into livestock fodder, which could increase the availability of feeds for ruminants throughout the year in both communal and commercial farming sectors.

1.2 Justification of the study

Given the scarcity of feed and the increasingly magnitude of bush encroachment in Namibia, there is a need for mitigating and coping strategies that are climate resilient, environmental friendly and innovative. Converting biomass from encroacher bushes into livestock fodder could be one potential way for increasing the availability of feeds for ruminants throughout the year, without competing with food production. De-bushing also creates space for increased grass cover and ultimately results in improved rangeland productivity (de Klerk, 2004; Hugos and Smit, 2005).

The concept of converting encroacher bushes to bush feed dates back as early as 1971 in Namibia (De-bushing Advisory Services, 2016), by some farmers that experienced bush encroachment. However, such efforts have been done on a trial-and-error basis with limited scientific knowledge on encroacher woody plant browse and their utilization as feed for livestock in Southern Africa (Moleele, 1998; Katjiua and Ward, 2007). Specifically, there is paucity of information on the nutritional quality of bush-based feeds, the optimal inclusion rate of the bush biomass materials in the rations, the possible adverse effects on animal health, quality of animal products and environmental impacts.

1.3 **Problem statement**

Animal fodder was reported in 2016 as the 8th largest imported good in Namibia, totalling 4bn N$ (NSA, 2016), which demonstrates the existing gap for the local production of animal fodder. In addition, long dry seasons coupled with frequent droughts also aggravate the feed shortage situation in the country (MAWF, 2012). The latest 2019 drought spell claimed lives of at least 90 000 livestock due to resultant deterioration of grazing and thirst (Shikangalah, 2020). Another compounding threat to livestock production in Namibia is bush encroachment and it is estimated that it has reduced the rangelands carrying capacity by two thirds (De Klerk, 2004; National Rangeland Management Policy, 2012).

Converting encroacher bushes to bush feed had proved to be a potential alternative feed resource in Namibia (De-bushing Advisory Services, 2016; Honsbein *et al*., 2017; Epafras, 2019). However, there is a knowledge gap on the potential quality of bush-based feed in terms of nutritional quality, digestibility, the possible effects on animal performance and products' quality (de la Puerta, 2017). Although most bush species are known for their high protein content (Mapiye *et al*. 2009; Makkar, 1999; Abdulrazak *et al*., 2000) of up to 26% w/w protein (Kamupingene *et al.*, 2004; Schneiderat, 2011), they also contain variable amounts of different anti-nutritional factors (ANFs) (Dube *et al*., 2001; Makkar, 2003). When high doses of ANFs up to 10–15% dry weight are consumed (Soetan and Oyewole, 2009), they can pose a risk to the animal's health, reduce the availability or utilization of nutrients and/or feed intake (Tadele, 2015). This ultimately reduces digestive efficiency and causes fluctuation in the expected performance of animals (Lange *et al*., 2000). It is therefore important to determine the ANFs in the four encroacher bush species because of their possible influence on the nutritional value on bush-based feeds and animal performance.

Determining the feeding value of encroacher bushes through *in vitro* or *in vivo* methods (Nousiainen *et al.*, 2003) is critical in order to provide guidance on the optimal incorporation of milled bush biomass in rations without compromising intake, growth, carcass characteristics and animal welfare. Finally, the production of greenhouse gases (GHG) particularly methane, by livestock and its impact on the environment is a worldwide concern because GHGs contribute to global warming (Theart, 2015). Hence, it is important to estimate methane production associated with the consumption of bush-based feed.

1.4 Objectives of the study

The general objective of the study was to evaluate the nutritional and feeding value of four abundant encroacher bush species (*Senegalia mellifera*, *Dichrostachys cinerea*, *Terminalia sericea* and *Rhigozum trichotomum*) as roughage sources in the diets of sheep.

The specific objectives of the study were to:

1. Determine the nutritional and polyphenol content of the four bush species (*Senegalia mellifera*, *Dichrostachys cinerea*, *Terminalia sericea* and *Rhigozum trichotomum*);
2. Determine the *in vitro* digestibility and methane production as well as *in sacco* neutral detergent fibre digestibility of the four bush species;
3. Evaluate the effect of feeding diets based on the four encroacher bushes on nutrients intake, digestibility and nitrogen retention of Damara lambs;
4. Evaluate effect of feeding diets based on the four encroacher bushes on feed intake and growth performance of growing Damara lambs;
5. Evaluate effect of feeding the four bush-based diets on the slaughter weight and carcass characteristics of Damara lambs.

1.5 Hypotheses of the study

1. H_{01}: The nutritional and polyphenol content of the four selected encroacher bush species do not significantly differ, despite the changes in plant physiological status in different seasons of the year.

2. H_{02}: The *in vitro* digestibility and methane production as well as *in sacco* neutral detergent fibre digestibility of the four selected encroacher bush species do not significantly differ, despite differences in agro-ecological zones where they come from and the season of harvesting;
3. H_{03}: Different bush-based diets fed to Damara sheep have no significant effect on their *in vivo* digestibility, nutrients intake and nitrogen balance, despite possible differences in chemical composition;
4. H_{04}: Growing Damara lambs fed bush-based diets do not significantly differ in feed intake and growth performance despite possible differences in nutritive values of the diets;
5. H_{05}: Different bush-based diets have no significant effect on the slaughter weights and carcass characteristics of Damara lambs despite possible differences in nutritive values.

1.6 **Significance of the study**

This research is anticipated to contribute to the knowledge of overall quality of bush-based animal feed, possible effects on the environment, as to guarantee the safety of the feed to animals and animal products from the bush-based feed to consumers. It will also have the following added benefits through the value addition interventions of bush biomass from encroacher bush species and bush control programmes at a national level: restoration of rangelands by increased carrying capacity and productivity; strengthen resilience of farming communities to drought and climate change effects by providing alternative sources of feed; sustainable local feed supply and possible substitution of imported feed; income generation and employment creation.

1.7 **Limitations of the study**

The selected encroacher bush species were restricted to certain agro-ecological zones and this posed logistical challenges in the processing of diets for the feeding experiments e.g. transportation of harvested bush materials to the feeding site due to limited financial resources. In addition, unavailability of some laboratory equipment and expertise for some analyses e.g. meat quality, structural analysis of polyphenols, *in vivo* methane and blood metabolites, which could not be done.

1.8 Delimitation of the study

Different alternatives were considered to ensure that the process of harvesting and transportation of bush biomass to the experimental site was not compromised. The candidate applied and was granted extra research sponsorship in Finland for six months to conduct proximate analysis, minerals, protein fractions, polypenols, amino acids, fatty acids, *in situ* NDF digestibility, *in vitro* OM digestibility and methane for late dry season and early rainy season samples, while at the same time being capacitated on the identified skills gaps.

CHAPTER 2

Literature review

2.1 Introduction

Most livestock in the country depend highly on natural rangelands for feed resources, which comprises of grasses and browse plant species. However, according to Shikangalah and Mapani (2020), about 45 million hectares of Namibian farmlands are being progressively taken over by massive growth of woody plants and/or bush encroachment. This results in loss of herbaceous vegetation on many rangelands and a decline in forage availability, while posing a further threat to livestock productivity (Archer *et al*., 2017). In addition, this condition is worsened by recurrent droughts often associated with heavy economic losses to farmers (Reid *et al*., 2007).

The aim of this review was therefore to describe, summarise and highlight the impact of bush species on livestock production, in order to promote research and development programmes on encroacher browse species as feed to livestock in Namibia.

2.2 An overview of sheep production in Namibia and its contribution to the economy

Livestock is the main farming practice in Namibia, with the majority of the producers in the southern parts of the country, farming commercially with sheep. Despite being drought-prone, Namibia has more than four million small ruminants, and ranks third in Southern Africa in sheep production after South Africa and Tanzania (Kahuika *et al.*, 2006). Sheep production activities in Namibia are concentrated in the more arid southern regions, especially Hardap and Karas regions, which account for 68% of the country's sheep population (Morris & Mare, 2013). Sheep can adapt to a wide range of climatic conditions and thrive well in dry areas where feed resources are limited (Erasmus, 2000).

There are well organized and established stud breeders associations for all the sheep breeds registered with the Namibian Stud Breeders Association. While Namibia has several breeds of sheep and goats for commercial trade, the Dorper sheep and Boer goat are the prime breeds. However, Damara, Van Rooy and Swakara sheep are also important breeds due to their adaptability to arid conditions. Swakara is a popular commercial sheep breed for pelt production. However, since the collapse of the Swakara industry in the early 1980's, the majority of sheep producers diversified into mutton production and within five years, the small ruminant industry changed from pelt production to mainly mutton production (Metzger and Rothkegel, 1990).

Namibia is a net exporter of mutton and Swakara pelts, with about 80% of all produce in the country exported to South Africa and the rest to the European Union (EU) markets (Oosthuizen and Laubscher, 2019). On the other hand, South Africa has been the main export destination for Namibian sheep as live animals. However, since the introduction of the Small Stock Marketing Scheme (SSMS) in 2004, live animal exports have been decreasing, for instance of the 820, 786 sheep marketed in 2018, live exports accounted for 55.5% (Meat Board, 2019).

2.3 Challenges and constraints to sheep production in Namibia

Commercial farming areas in Namibia are mainly faced with the problem of bush encroachment (de Klerk, 2004). This is a phenomenon associated with invasion/thickening of undesired woody species and reduction of palatable grasses. Recent assessments indicated that Namibia is affected by bush encroachment on a massive scale, with approximately 45 million hectares of Namibia's agricultural land being encroached (Shikangalah and Mapani, 2020).

Since the majority of livestock in the country depends highly on the natural rangelands for feed resources, bush encroachment results in loss of herbaceous vegetation and reduces the carrying capacity of many rangelands, while posing a further threat to livestock productivity (Bovey, 2001). In addition, this condition is worsened by recurrent droughts often associated with heavy economic losses to farmers because of animal weight and condition losses, reduced reproduction and increased mortality rates (Sweat and Burke, 2000).

On the other hand, small ruminant productivity in the communal areas also remains marginal due to various constraints including poor management, infectious animal diseases, low genetic potential, poor nutrition, stock theft, limited marketing infrastructure, limited natural water resources, threats of poisonous plants and limited grazing land (Directorate of Veterinary Services (DVS), 2012). In the commercial farming areas, the export restriction policy introduced through the Small Stock Marketing Scheme in 2004, appears to have also caused a dramatic decline in sheep population and a significant shift from live exports to slaughtering in Namibia (Oosthuizen and Laubscher, 2019). Feed shortages, in particular brought about by recurrent droughts, are a major constraint to the livestock industry. Hence, there is a need to develop and transfer sustainable interventions such as value addition of non-conventional feed to fill seasonal feed gaps in order to avoid environmental deterioration, improve livestock production and improve livelihoods.

2.4 Bush encroachment and the most encroaching species in Namibia

Bush encroachment refers to the invasion and/or thickening of woody species to the detriment of herbaceous (grassy) layer (de Klerk, 2004). A standardized "bush equivalent" (BE) which refers to a 1.5 m high bush is commonly used in Southern Africa, including Namibia (Smit *et al*., 2015), to assess the severity of the bush thickening. Smit *et al*. (2015) suggest that, as a rule of thumb, a density of encroacher bush equivalent per hectare (BE/ha) that exceeds twice the long-term average rainfall (mm per year) represents bush encroachment. Areas affected by bush encroachment in Namibia recorded densities which vary between 2,000 and 24,000 bushes per hectare (Smit *et al*., 2015), with 5,000 to 6,000 BE/ha, thorn bushes being impenetrable. The density of bush encroachment by dominant encroacher species in various parts of Namibia is presented in Figure 2.1.

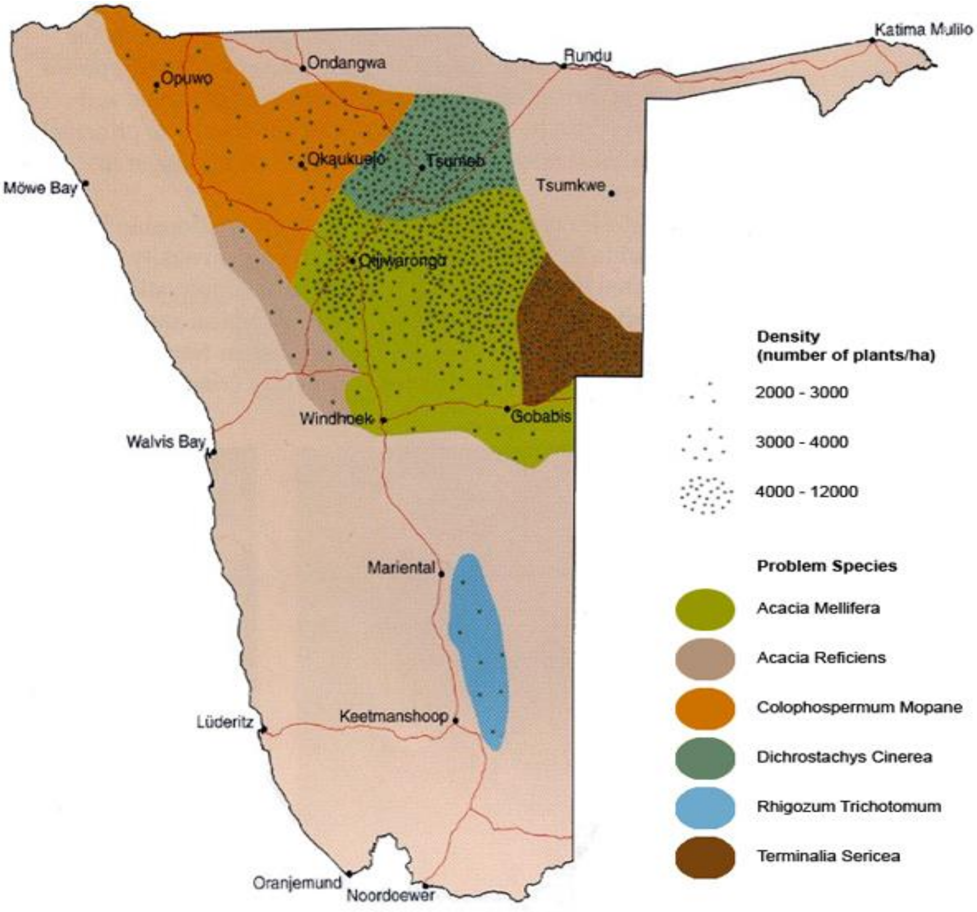

Figure 2.1 Occurrence of dominant encroacher species in Namibia and their density per hectare Source: de Klerk (2004)

According to de Klerk (2004), the main species responsible for the encroachment problem in affected areas of Namibia are: *Colophospermum mopane*, *Acacia reficiens*, *Dichrostachys cinerea*; *Senegalia mellifera*, *Terminalia sericea* and *Rhigozum trichotomum.*

2.5 Descriptions and characteristics of selected encroacher species

2.5.1 *Senegalia mellifera* (**Black thorn)**

Family: Fabacea or Leguminosae. Curtis and Mannheimer (2005) describes the species as a single stemmed, v-shaped shrub with round crown and height usually ranging from 1 - 8 m. The leaves are structured with pinnae pairs, each with single leaflet. Thorns are hooked and blackish, while flowers are short roundish with sparks. Pods are thin, paperish and straw coloured at maturity. The bush is common to abundant in central Namibia, and appears in a broad band across western Namibia and occasionally found in the north-west of Namibia.

2.5.2 *Dichrostachys cinerea* (**Sickle bush**)

Family: Fabaceae or Leguminosae. It is an aggressive encroacher species, which usually grows as a multiple stemmed deciduous shrub or small tree. It has small leaflets, dark grey bark with longitudinal fissures, spikes with pink flowers at the base and yellow apical. The pods are dark brown, hanging in clusters, and are used as fodder for livestock. It is found in a wide variety of habitats but more common to abundant on plains and hill slope, especially on the north-central plateau (Curtis and Mannheimer, 2005).

2.5.3 *Terminalia sericea* (**Silver Cluster-leaf**)

Family: Combretaceae. As described by Curtis and Mannheimer (2005) is a medium-sized tree with branches growing horizontally, giving the crown a layered appearance. The leaves cluster towards ends of branchlets, pale silverly-green in colour with silky hairs. Flowers are cream to pale yellow, in axillary spikes, while fruits are pink to rose-red or brown when dry. It is widespread and commonly found in sandy areas of Namibia, mostly north-east and central-east. It is mostly browsed by cattle and game.

2.5.4 *Rhigozum trichotomum* (**Three-thorn bush**)

Family: Bignoniaceae. A rigid, deciduous shrublet, with simple dark green leaves that are spirally arranged or clustered on dwarf shoots, often glandular or sticky looking. Its young branchlets split in threes at growth points. The flowers are solitary, trampet-shaped, white or flashed pale pink in colour, while fruits are dry, papery capsule and tend to split early. The shrub is commonly found on the southern and eastern part of the Namib Desert, but also scarcely found further north of the country (Curtis and Mannheimer, 2005). This plant is mostly browsed by goats.

2.6 Potential edible biomass and accessibility of browse by ruminants

Browse plants provide substantial amount of biomass from leaves and small twigs as well as pods for ruminants to meet their nutrient requirement during periods of feed shortage in the year (Kamalak, 2006; Ngwa *et al.*, 2011). Despite their importance, few studies have integrated browsing resources in their rangeland resource assessments in Southern Africa (Dekker and Smit, 1996; Moleele, 1998; Mphinyane, 2001; Kamupingene and Abate, 2004; Dube *et al*., 2006). Such an understanding is necessary to evaluate the potential browse capacity in order to match the browse supply to browser demand, which is central to sustainable utilisation and the monitoring of vegetation health (Penderis, 2012). The most widely recognised browse prediction model in southern Africa is the BECVOL (Biomass Estimates from Canopy VOLume) model developed by Smit (1989). According to Penderis (2012), this method proved not only useful in the estimation of browsing capacity, but also in other management areas, such as the ability of the area to sustain fire and the monitoring of bush encroachment and tree growth.

Regardless of the browsing capacity, browse accessibility may still present one of the challenges to their efficient utilization by ruminants. According to Le Houérou (1980), animals have access to what they can reach and without human intervention only part of the feed available can be eaten by the animals. Thus, Dambe *et al*. (2015) concurred with Le Houérou (1980), of height as a hindrance to consumption of most tree leaves until they have fallen naturally to the ground where animals find them. The implication is that by then, such leaves would likely have experienced a considerable drop in nutritional value and would have low feeding value to animals.

Furthermore, some areas with heavily bush encroached sites may have impenetrable thickets of usually *Vachellia* and *Senegalia* (formerly called *Acacia)* species and/or *Dichrostachys cinerea* which may render such browse inaccessible to livestock (Dambe *et al*., 2015). Other accessibility factors include developed defence mechanisms by browse such as thorns and fibrous foliage that assist their survival and protect their crown from grazing ruminants (Aruwayo and Adeleke, 2019). According to Bergström (1992), thorns and spines as seen in *Acacia* spp. may not only prevent animals from feeding, but they certainly also reduce bite size and biting rate. Therefore, to improve the utilization of woody browse plants especially in encroached areas, human intervention is necessary by selective thinning of woody vegetation and then processing the edible biomass (leaves and twigs) into animal fodder.

2.7 **The role of browse as feed resources for ruminants**

Browse resources (shrubs, bushes and trees) are of utmost importance in arid or semi-arid regions, due to their main characteristics of being rich in protein and phosphorus throughout most of the dry season (Blümmel *et al*., 1999). In contrast to grasses, shrubs and trees generally shoot before the first rains; and shoots retain their nutritive value when other feed resources are depleted both in quantity and quality (Bergström, 1992). Thus, they are a possible compensation of a deficit caused by the energy-poor dry herbaceous forage during the dry season or where there is little grass available (Dube *et al*., 2001; Kamupingene *et al*., 2004).

Additionally, browse resources are more stable and less prone to additional over-exploitation (Peel *et al*., 1998) and their ability to remain green for a longer period could also be attributed to their deep root systems, which enable them to extract water and nutrients from deep in the soil profile. Blümmel *et al*. (1999) also emphasised that browse can as well be of importance where prices of feed supplements are prohibitive, hence, giving farmers an alternative feed for survival during the drought period.

Contrary to natural browsing, a number of farmers in Namibia have been reported to be producing bush-based animal fodder utilizing predominant encroacher woody species such as *S. mellifera* (Black thorn), *T. sericea* (Silver cluster-leaf), *C. alexandri* (Gobbabos), *R. trichotomum* (Driedoreng) and *D. cinerea* (Sickle bush) (Honsbein *et al*., 2017) in response to the recurrent droughts in Namibia. The process of bush-based feed production involves harvesting and chopping of leaves and small twigs with a diameter of less than 20 mm ("broom stick size"), milling them in hammer mill and mixing the milled bush materials with different supplements such as molasses and camelthorn pods. This initiative seems to be promising and gaining momentum in different regions of the country, especially where there is bush encroachment.

The concept of converting encroacher bushes to bush feed dates back to as early as 1971 in Namibia (De-bushing Advisory Service (DAS), 2016), by some farmers that experienced bush encroachment. However, such efforts have been done on a trial-and-error basis with limited scientific verification on the quality of produced bush feed. This could be in terms of nutritional quality, the optimal inclusion rate of bush biomass in the rations, the possible effects on animal health and performance, animal products' quality and any environmental impact. Hence a need exists to fill knowledge gaps in those areas to realise the full potential of bush-based feed.

2.8 Nutritional and feeding value of selected encroacher woody browse species

Penderis (2012) described browse chemical analysis, animal preference, intake, digestibility and production of animals on browse as factors that are tightly linked and suggested that they need to be considered together, in trying to improve the understanding of potential browse species. Mtengeti and Mhelela (2006) also shared the same sentiment that any method used to screen potential browse species must consider both the preference of browsing animals and laboratory analyses to verify the nutritional quality of the browse plants. In addition, Merga and Becho (2016) also indicated that the feed value is the potential to supply the nutrients required by an animal both quantitatively and qualitatively in order to support a desired type of production. Therefore, the feeding value of a feed resource is influenced by its chemical composition, digestibility, physical nature, palatability, associative effects when given in a ration and the physiological status of the animals.

2.8.1 Chemical composition

According to Cooper *et al*. (1988), the chemical factors of browse fall into three categories: (a) nutrients, including protein and various mineral elements; (b) fibre, including cellulose, hemicelluloses and lignin, which influence physical toughness as well as digestibility; (c) plant secondary metabolites, which may function as toxins or to reduce the digestive availability of nutrients.

There are many reports of chemical analysis of browse plants in general but few were found on selected encroacher browse plants and are presented in Table 2.1. Many of these studies were done either on leaves alone or leaves and twigs. However, the data presented shows that the available information on their chemical analyses is scanty, highly variable and either done on incomplete proximate analysis or on crude protein content alone. The high variability in chemical constituents could be attributed to the plant part, stage of maturity of the plant, harvesting procedure, leaf-stem ratio in the forage, season, location and type of the browse plant.

The available data presented in Table 2.1, indicates that the crude protein (CP) content of all selected browse species ranged between 8.25% in *R. trichotomum* (Marius, 2016) and 20.34% in *S. mellifera* (Theart *et al*., 2015). The reported CP contents of all four species (*S. mellifera, T. sericea, R. trichotomum,* and *D. cinerea*) were sufficient to meet the maintenance requirements of ruminants (7%) as indicated in the NRC (2000, 2001, 2005) guidelines.

There are protein evaluation methods of ruminant feeds such as *in vivo, in situ* and *in vitro* which distinguish between the protein fractions that are degraded in the rumen and may be converted to microbial crude protein, and undegradable or by-pass protein which is more or less digestible in the intestine (Spanghero *et al*., 2010; Mahesh *et al.*, 2017; Acar, 2018; Acar *et al*., 2019). Acar *et al*. (2018) highlighted that the conventional *in vivo* method which is thought to be accurately reflecting the feeding value and protein degradability of forage and total rations, is labour-intensive and expensive. Therefore, other alternative methods such as *in situ* Nylon Bag Technique (NBT) and *in vitro* Cornell Net Carbohydrate and Protein System (CNCPS) have become increasingly popular and can be used to generate accurate information on protein fractions of feeds for ration formulation.

Typically, tropical forages are lower on soluble protein and the amount of undegradable plant protein escaping the rumen depends on the digestibility of the forage and the chemical and physiological characteristics of the protein (McSweeney *et al*., 1999). In the review work by Topps (1992) on some legume shrubs and trees, it was concluded that the insoluble fraction of their total protein which is not degraded in the rumen nor digested in the gut, varies in relative amount among different parts of the plants. However, the same work showed that a large fraction (0.77-0.87) of their CP, irrespective of plant part, was degraded in the rumen. In view of this, knowledge of potential rumen protein degradability of milled bush biomass is limited and should be considered in assessing their nutritive value.

In terms of fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) seem to be variable in presented studies even within the same species. The high quality of some browse species in minerals such as calcium and phosphorus throughout the dry season (Blümmel *et al*., 1999), is well appreciated as one of their main characteristics. The available information on mineral composition of selected browse species is rather sketchy and fragmentary covering some macro-elements; information on trace elements such as Cu, Mn, Zn, I and Co was not available. The reported data on selected species indicated that Ca levels vary from 0.65 to 1.13% DM, while P contents vary from 0.09 to 0.13% DM, mainly in leaves. The available information on EE from the presented studies was scanty to make a meaningful conclusion of their contents in either leaves alone or leaves and twigs.

On the other hand, the chemical composition of milled bush biomass from some Namibian encroacher species contained variable amount of CP ranging from 1.57 to 15.5% DM depending on the species and season (Honsbein *et al*., 2018; Epafras, 2019). Previous research work by Honsbein *et al*. (2017) also indicated that milled bush biomass from some Namibian encroacher species contained substantial amount of cell wall carbohydrates in the form of NDF, which ranged from 43.33 to 58.79% depending on the species and season of harvest. Neutral detergent fibre includes hemicellulose, cellulose and lignin as major components (Van Soest *et al*., 1991a) of which lignin is generally accepted as the primary component responsible for limiting digestion on cell wall carbohydrates in the rumen. Due to variability of NDF degradation in the rumen and its influence on animal performance (Jančík *et al*., 2008), the knowledge of NDF digestibility on milled bush is critical for their effective use in feeding ruminants and should be considered.

The *in sacco* nylon bag technique (NBT) is commonly used to estimate the NDF digestion by 12 days ruminal incubation of forage samples in nylon bags using ruminally cannulated animals and the NDF fraction disappearing after that ruminal incubation period is referred to as potentially digestible NDF (pdNDF) (Nousiainen *et al*., 2003; Jančík *et al.*, 2008; Harper and McNeill, 2015; Teimouri Yansari, 2017; Soufizadeh *et al*., 2018). Therefore, Traxler *et al*. (1998) suggested that it is important to determine the indigestible NDF (iNDF) and exclude it from the estimation of forage energy content because it does not contribute energy to the animal.

Table 2.1 Chemical composition (%DM) of the selected encroacher browse species

Browse specie	**Plant part**	**DM**	**Ash**	**OM**	**EE**	**CP**	**NDF**	**ADF**	**ADL**	**Ca**	**P**	**Location**	**Reference**
Senegalia mellifera	Leaves	-	7.3	-	-	19.2	57.0	32.5	17.5	-	-	Tanzania	Nassoro (2014)
		94.47	6.59	87.88	-	-	33.60	24.77	-	0.72	0.09	Namibia	Marius (2016)
		-	-	93.0	-	19.4	26.9	19.2	7.7	1.13	0.09	Kenya	Abdulrazak *et a*
		89.89	-	93.23	-	14.95	39.94	32.22	9.48	-	-	Kenya	(2000)
	Leaves and twigs	-	-	-	-	11.63	-	-	-	-	-	Botswana	Osuga *et al.* (2008)
		92.22	8.41		4.03	20.34	37.36	30.21	8.01			S.Africa	Aganga *et al.* (1998)
													Theart (2015)
Dichrostachys cinerea	leaves	94.55	14.48	-	1.92	14.66	-	-	-	-	-	Namibia	Kasale (2013)
		94.54	5.95	88.59	-	11.7	48.13	34.57	-	0.65	0.13	Namibia	Marius (2017)
	Pods	92.18	6.32	-	0.8	16.64	-	-	-	-	-	Namibia	Kasale (2013)
	Leaves and twigs	91.02	4.05	-	2.60	14.15	50.89	37.68		-	-	S.Africa	Theart (2015)
Terminalia sericea	Leaves	-	-	-	-	11.6	-	-	-	_	-	Namibia	Kamupingene *et a*
		94.56	5.58	89.08	-	10.36	41.79	39.22	-	0.75	0.09	Namibia	(2004)
													Marius (2016)

	Leaves and twigs	92.45	5.13	-	6.37	9.74	52.01	44.57	10.53	-	-	S. Africa	Theart (2015)
Rhigozum trichotomum	Leaves	93.39	6.42	86.92	-	8.25	39.85	20.95	-	0.91	0.11	Namibia	Marius (2016)

DM-Dry Matter; OM-Organic matter; EE-Ether Extract; CP-Crude protein; NDF-Neutral detergent fibre; ADF-Acid detergent fibre; ADL-Acid detergent lignin; Ca-Calcium; P-Phosphorus

2.8.2 Amino acids and fatty acids composition

Boisen *et al*. (2000) emphasised that despite the protein degradability of forages in the rumen, the most important single factor affecting the efficiency of protein utilization is the profile of digestible essential amino acids entering the small intestine. According to McSweeney *et al*. (1999), a well-balanced supply of amino acids is required but this is often not the case with roughage diets which can be limiting in several amino acids including methionine, cysteine, lysine, histidine and possibly threonine, valine and isoleusine.

Although, the microbial protein synthesized in the rumen are used to meet the amino acid requirements of ruminants, the dietary protein that escapes degradation also makes a significant contribution to the overall supply of amino acids. Therefore, McSweeney *et al*. (1999) and Acar *et al*. (2019) suggested that knowledge regarding the amino acids profile is crucial in accurately formulating rations to ensure the maximum amount of synthesised microbial protein in the rumen and the availability of the remaining undigested plant protein for digestion and absorption in the intestines. The amino acids composition of milled bush biomass from encroacher bushes is scarce compared to that for grasses and legumes (Tedeschi *et al*., 2001; Edmunds *et al*., 2013).

In addition to amino acids composition, Acar *et al*. (2019) emphasised the importance of knowing the total fatty acids (FAs) and fatty acids composition of the dietary feedstuffs, which are subsequently linked with consumer-desired FA composition of ruminant meat and dairy products (Goossen *et al*., 2018). Some studies have evaluated the FAs on grasses and/or legumes in other parts of the world (Boufaïed *et al*., 2003; Glasser *et al*., 2013; Goossen *et al*., 2018; Acar *et al*., 2019) but limited information exists on FAs of browse.

There is a growing interest in the nutrient composition of the diet, which can change the fatty acid profile of meat and milk to make them more attractive for health reasons. Alfaia *et al*. (2009) and Nuernberg *et al*. (2005) reported that including forage in the diet of beef cattle enhanced the n-3 fatty acid concentrations in meat because forages are a good source of C18:3n-3. Similarly, Khosravi *et al*. (2018) also reported the positive effects of forage based silage on polyunsaturated fatty acids of milk.

The nature and composition of forages influence FA metabolism in the rumen; it is thus of interest to study the factors that modify FA in forages (Glasser *et al*., 2013). The FAs of forages are affected by numerous factors such as plant species and variety, climate, day length, rainfall, fertilization, stage of growth and storage methods (Boufaïed *et al*., 2003; Acar *et al*., 2019). Goossen *et al*. (2018) reported a decrease in a-linolenic acid (ALA) and saturated fatty acids (SFA) content in maturing forage grasses. In addition, the processing method such as silage making or haymaking has also been reported by Acar *et al*. (2019) to influence the content of FAs of forages. On the other hand, the same authors highlighted that even though hays contain relatively low level of FAs, they are the cheapest and often the major source of unsaturated FAs in ruminant diets. In light of this, knowledge of the factors that influence FAs composition of the browse shrubs and trees, could therefore, help farmers to optimize harvest conditions and time, thereby contributing to the improved quality of the forages.

Overall, due to limited information on most chemical constituents of the selected encroacher browse species, further work is certainly needed to improve knowledge on their nutritional value for efficient utilization as promising substitutes for fodder to improve the nutritional status of animals.

2.8.3 **Plant Secondary metabolites**

Plant secondary metabolites, commonly known as anti-nutritional factors (ANFs) are substances that when present in feed, reduce the availability or utilization of one or more nutrients and/or feed intake of plants or plant products used as animal feeds (Tadele, 2015). Browse plants contain ANFs that vary widely in structure and concentration (Soetan and Oyewole, 2009). Typical responses to ANFs when consumed by ruminants and non-ruminants are lower feed intake, protein and dry matter digestibilities, live weight gains, milk yield and wool growth (Mueller-Harvey, 2006). However, their effects usually depend on the amount of the ANFs consumed and will also vary with the animal species (Bwire *et al*., 2004; Mpofu *et al*., 2016; Brown and Ng'ambi, 2019). This may imply that different levels of ANFs in plants might affect animals differently and thus their characterization is of importance in determining their safety in ruminant feeding (Atiku *et al*., 2016; Yacout, 2016).

The ANFs which have been implicated in limiting the utilisation of shrubs and forage trees include polyphenolic compounds (tannins, lignin), glycosides (saponins, cyanogens), flavonoids and alkaloids (Akande *et al*., 2010; Tadel, 2015; Atiku *et al*., 2016). According to Tadele (2015), a single plant may contain two or more ANFs from different groupings, which could add to the difficulties of managing their harmful effects.

2.8.3.1 Tannins

The most widely occurring secondary metabolites in most species of browse trees and bushes are tannins (Makkar, 2003). Tannins are usually divided into two subgroups: hydrolysable and condensed tannins, which differ in nutritional and toxic effects (Patel *et al*., 2013). Hydrolysable tannins have a central carbohydrate core, the hydroxyl groups of which are esterified to various phenolic carboxylic acids (Patra and Saxena, 2010). This group of tannins is easily hydrolysed to give glucose or a polyhydroxy alcohol and the various phenolic acids (Makkar, 2003; Mlambo *et al*., 2011). The condensed tannins are often referred to as proanthocyanidins because they produce red anthocyanidins when heated in acid and they have more profound digestibility-reducing effect than hydrolysable tannin (Akande *et al*., 2010). Tannins are considered to have both beneficial and toxic effects to animals depending on their type and concentration, as well as on the type and age of animals (Gxasheka *et al*., 2015).

Detrimental effects of tannins are commonly associated with reduction in ruminal protein digestion (Silanikove *et al*., 2001). Most browse are protein rich, up to 26% w/w protein (Kamupingene *et al*., 2004; Schneiderat, 2011), but their digestibility is restricted by relatively high levels of endogenous tannins. For example, condensed tannins reduce the bioavailability of protein in browse plants by interfering with their digestion and metabolism, especially when consumed in high quantities of 10–15% dry weight (Cooper *et al*., 1988; Bensalem *et al*., 2004; Soetan and Oyewole, 2009; Makkar, 2010; Mancilla-leytón *et al*., 2014). The mechanism underlying the effects of tannins arises from their ability to form strong H bonds with the nutrients resulting in inhibition of digestive enzymes and rumen microbial activities (Tadele, 2015) and their effects can increase with the increase of the tannins molecular weight (Yacout, 2016). This may indicate that the binding strength in tannin–protein complexes is an important factor in ruminal protein digestion.

However, even though protein digestibility tends to be the most reduced, other nutrient constituents such as carbohydrate, starch and cell wall digestibility can also be affected. In addition, Gxasheka *et al*. (2015), Tadele (2015) and Yacout (2016) indicated that high tannin contents are also associated with reduced overall digestibility, poor feed palatability, decreased feed intake and reduced growth rate or loss in weight. Other detrimental effects of tannins in the form of poor nutrient utilization and decreased iron absorption and digestive upsets in the animals may also occur (Silanikove *et al*., 2001; Yacout, 2016).

On the other hand, Egbuna and Ifemeje (2015) reported that tannins are one group of the ANFs, for which beneficial effects in animals have been widely studied. The main benefit of tannins in ruminant nutrition is associated with their effect on protein digestion. Mueller-Harvey (2006) has reported that when tannin levels do not exceed 5% dry weight, tannins can reduce the amount of protein that is digested in the rumen and enhance the amount of protein that is available for digestion in the small intestine.

Max *et al*. (2005) illustrate that tannins are another way to protect proteins against excessive degradation in the rumen, by forming reversible complexes with proteins. They further explained that these complexes are not degraded at pH values present in the rumen, but they disintegrate at pH values of the abomasum and small intestine. This happens when tannins bind feed proteins to form feed protein-tannin complexes, which are resistant to rumen microbial degradation (Mueller-Harvey, 2006). The author further highlighted that this process shifts the form of protein to what has been referred to as ruminal escape protein or rumen undegradable protein. This can be an advantage in reducing excess degradation of high quality protein in the rumen and helps in increasing the amount of rumen undegradable protein, which is finally made available to the host animal for supplying essential amino acids (Simbaya, 1997).

Other benefits of tannins in animals feeding include: reduction of parasite burden, bloat prevention, increased quality of animal products, reduction of methane emission into the environment and defaunating the rumen (Ammar *et al*., 2004; Piñeiro-Vázquez *et al*., 2015). Tannins were also found to reduce proteolysis during ensilage (i.e. they can protect protein during ensiling) (Yacout, 2016). This suggests that proper management of tannins in bush-based feeds by establishing optimal levels could enhance their feeding value, while yielding various benefits.

2.8.3.2 Saponins

Saponins are glycosides which occur in a wide range of plants and they are characterized by a bitter taste and foaming properties (Kumar *et al*., 2009; Tadele, 2015).

Their anti-nutritional properties seem to be related to their ability to form complexes with sterols, in particular those in membranes of animal cells resulting in increased permeability of the intestinal mucosa (Yacout, 2016). In ruminants, saponins may not retard the growth because they can undergo bacterial degradation in the rumen, but they have been implicated in causing bloat (Kumar, 1991) and they are differentially toxic to rumen protozoa (Teferedegne, 2000).

2.8.3.3 Cyanogens

Cyanogens are glycosides of sugars and cyanide containing aglycone. They can be hydrolysed by enzymes to release free hydrogen cyanide (HCN) (Kumar, 1991). The HCN is absorbed and rapidly detoxified in the liver by the enzyme rhodanese which converts cyanogenic glycocides (CN) to thiocyanate (SCN) (Tadele, 2015). Excess cyanide ions cause cyanide toxicity, when they combine with haemoglobin in blood and inhibit respiratory enzymes, ultimately causing death.

In ruminants, the hydrolytic reactions can take place in the rumen by microbial activities, and this process makes ruminants more susceptible to HCN than non-ruminants. In the rumen, HCN is converted to thiocyanite using available sulphur and thiocyanite is absorbed and excreted (Norton, 1994).

2.8.3.4 Alkaloids

Alkaloids are present in many plants whereby they are thought to serve as a chemical defence against herbivory and often have a bitter taste (Teferedegne, 2000). Alkaloids are oxidized in the liver resulting in metabolites, such as dehydrosparteine, which are responsible for the observed toxicity (Atiku *et al*., 2016). The level of toxicity is influenced by the structure of the alkaloids and there is a high degree of variation in the ability of different animal species to deal with these compounds.

Alkaloids are considered to be anti-nutrients because of their action on the nervous system, disrupting or inappropriately enhancing electrochemical transmission. For instance, consumption of high tropane alkaloids will cause rapid heartbeat, paralysis and lead to death (Soetan and Oyewole, 2009). The toxic effects of alkaloids and their metabolites also stimulate copper uptake by liver cells thus leading to copper toxicity (Tadele, 2015). According to Yacout (2016), some alkaloids are also reported to cause infertility in animals.

2.8.4 **Methods to reduce the detrimental effects of Anti-nutritional factors**

Many feed processing techniques have been used as possible means to effectively eliminate or minimize different ANFs to levels that can be tolerated by animals. These include drying (sun or oven), chopping, grinding, milling, pelleting, heat (steam) treatment, ensiling, ammonia/urea treatment, biological treatment (enzymatic and fungal degradation) and chemical treatments such as PEG (Mueller, 2001; Makkar, 2003).

Although most of the above treatment methods or processing techniques for anti-nutritional factors were found to be effective, successful adoption has been relatively low based on unfavourable cost to benefit ratio (Yacout, 2016).

2.9 **Digestibility**

Fadiyimu *et al*. (2011) emphasised that even though proximate analysis does not give a full indication of the nutritive value of a feed, it provides clues in research on browse plants of potential value for further *in vitro* or *in vivo* digestibility studies. Digestibility (dry matter digestibility and other nutritional constituents) of foliage is one of the measures used to describe the nutritive value of foliage (Gebeyew *et al*., 2015).

2.9.1 ***In-vitro* digestibility and *in sacco* degradability methods**

The nutritional value of fodder plants can be estimated with adequate precision from *in vivo* digestibility (Getachew *et al*., 1998). However, due to the expense and time required to conduct animal trials, alternative biological procedures i.e. *in vitro* and *in situ/in sacco* techniques to predict organic matter digestibility have been developed. *In vitro* methods have also been used to determine the digestibility of protein (Shayo and Ude Ân, 1999; Barchiesi-Ferrari *et al*., 2011; Mahesh *et al*., 2017; Cömert Acar, 2018) and neutral detergent fibre (Jančík *et al*., 2008; Spanghero *et al*., 2010).

The *in vitro* gas production technique has been used to simulate ruminal fermentation of feed and feed stuffs (Getachew *et al*., 1998) for decades. The *in vitro* technique is a relatively simple method for screening and evaluating feed resources as large numbers of samples can be incubated and analysed at the same time (Makkar, 2002; Makkar, 2010). Besides, the methods have the advantages of being less costly, less time consuming, good reproducibility and also correlate well with values measured from *in vivo* trials (Getachew *et al*., 1998). The *in vitro* gas production technique has also been applied successfully in deriving the organic matter digestibility and metabolisable energy of feeds (De Boever *et al*., 1988). The effect of secondary metabolites such as tannin or saponins on rumen fermentation (Rubanza *et al.*, 2005) and methane production have also been evaluated *in vitro* (Makkar, 2010; Gemeda and Hassen, 2015; Macome *et al*., 2017; Melesse *et al*., 2019).

On the other hand, *in sacco* NBT is also widely used and forms the basis of many feed evaluation systems. The NBT described by Abdulrazak *et al*. (2000) and Tolera *et al*. (1997) for the determination of the degradation and digestibility of feedstuffs in the rumen at various incubation periods can be used to screen feeds at the initial stages of assessing their nutritive values. It has been used to determine rumen degradation of protein, organic matter and starch, and it is also the most common method used to determine NDF digestion or the content of indigestible NDF (iNDF) (Jančík *et al*., 2008). For example, the iNDF is determined *in sacco* by incubating nylon bags with feed samples in the rumen of cannulated ruminants (cow, sheep or goats) for 240 days (Jančík *et al*., 2008). Like other feed characteristics, rumen degradability of browse species varies widely and these variable effects could be attributed to the type of species, season, proportion of leaves to stem, its environment, management practices and nutritive value (Larbi *et al.*, 1997; Abdulrazak *et al*., 2000; Bouazza *et al*., 2012; Belachew *et al*., 2013).

2.9.2 *In vivo* digestibility and nitrogen retention

Feed evaluation methods involve the determination of chemical composition and digestibility. Although methods using rumen fluid (*in vitro*) have been widely and successfully used to determine forage OM digestibility (OMD) (Nousiainen *et al*., 2003), the *in vivo* digestion trials still remain the more reliable and accurate measure of digestibility because it takes into account the biological effects of the animals. Barchiesi-Ferrari *et al*. (2011) further indicated that *in vivo* data must always be considered as a reference method in the search for any reliably, accurate and precise digestibility predictive approach.

In relation to the above, nitrogen (N) is one of the limiting factors for feed intake and digestibility in ruminants and an essential nutrient necessary for efficient microbial growth in the rumen (Mlay *et al*., 2003). Mlay *et al*. (2003) further indicated that an adequate N supply to the rumen microbes is very important to obtain maximal rate of digestion of carbohydrates supplied with the feed as well as a high microbial protein synthesis. However, potentially high levels of ANFs such as condensed tannins reduce the bioavailability of protein in browse plants by interfering with their digestion and metabolism (Cooper *et al*., 1988; Bensalem *et al*., 2004; Makkar, 2010; Mancilla-leytón *et al*., 2014). Hence, it is important that reliable methods are used to determine quality traits of browse, such as nitrogen (N) retention to assist with the formulation of balanced ruminant rations (Barchiesi-Ferrari *et al*., 2011).

Increased N retention of 3.7 g N/day in ruminants as a result of being supplemented with fruits of some browse plants such as *Acacia* species and *D. cinerea* (Mlambo *et al*.,2004; Smith *et al*., 2005; Yayneshet *et al*., 2008) has been reported. However, it appears that the processing methods of fruits such as grinding or treating with PEG yielded varied results in those studies. Similarly, positive N retention values were also observed by Becho (2016) for some browse plants such as *leucaena* and *acacia* species, but a decreasing nitrogen retention was observed as the level of leaf replacement increased in the untreated *acacia* leaf meal diets while increasing trend was observed in treated leaf meal diets.

2.10 **Preference and intake of selected browse by ruminants**

The selected browse species were reported to be among the most preferred browse by ruminant livestock (Bester, 1999), although they were also being classified as potential problematic species that thicken under certain condition to encroach rangelands (de Klerk, 2004; Smit *et al*., 2015). Katjiua and Ward (2007) share the same sentiment with respect to *T. sericea*, which they indicated was regarded as a major encroaching species by range ecologists. While, pastoralists in a semi-arid region of Namibia regard this woody species as an important component of cattle diet and among the most preferred, particularly during the hot-dry season or during drought. However, they concluded that, cattle generally preferred browse with high crude protein and phosphorus content but avoided browse with high fibre content. On the contrary, Kamupingene *et al*. (2004), observed the opposite with *T. sericea* being the less preferred species compared to *Acacia reficiens* and *Acacia hebeclada* which were most preferred by goats in communal areas of Omaheke region of Namibia.

Another study by Kasale (2013) revealed that *D. cinerea* was among the most preferred browsable plants by cattle in Zambezi Region, Namibia. Osuga *et al*. (2008) observed that physical features of browse such as the spines did not appear to deter feeding, when goats and sheep browsed on *S. mellifera*, which suggests that the spines may have little influence in the preference of browse foliage. However, they further argued that the differences in preference may change depending on the adaptation of animals or large variation of their nutritional status through the year. This is because some browse species that are least preferred during periods when fodder availability is high could be relished during periods when available feeds are scarce. Furthermore, Mbatha and Bakare (2018) added that poor management practices like overstocking cause disappearance of palatable plant species through overgrazing and/or over-browsing. Hence, animals would have no other alternative but to select the remaining unpalatable plant species within a piece of land to get nutrients that meet their requirements for maintenance, growth and reproduction.

Different observations by Ngwa (2002) showed that the level of ANFs such as saponins and tannins may also affect the palatability of forages and hence preference by the animals. Therefore, all these studies reveal that having a variety of browse species in farming systems would ensure year-round availability of fodder for increased animal productivity.

2.11 Growth and meat characteristics of livestock supplemented with browse

The interest in the use of browse plants as animal feed has advanced with different studies conducted using different parts of shrub species (small twigs, leaves and pods) as supplement for animal nutrition. Gusha *et al*. (2014) observed positive growth in indigenous goats fed some browse legumes (*Acacia angustissima, Calliandra calothrysus* and *Leucaena leucocephala*) typically found in Zimbabweas supplement to mature pasture hay. In another study, Moyo *et al*. (2012) reported that crossbred Xhosa goats supplemented with *Moringa oleifera* leaves had positive growth with average daily gains of up to 103 g/day. Becho (2016) also observed an increase in average daily gain of goats fed treated *Acacia tortilis* leaf meal as a replacement for concentrate meal. In a study done on Small East African goats, Rubanza *et al*. (2007) observed positive growth of up to 157.1 g/day when they were fed leaf meals of different shrub species (*Vachellia* (formerly *Acacia*) *nilotica*, *V. polyacantha* and *Leucaena leucocephala*) as dry season supplement to native pasture hay basal forages.

Apart from studies on goats, Mapiye *et al*. (2011) demonstrated that supplementation with *V. karroo* leaf meal improved growth performance, carcass traits and the fatty acid composition of beef from Nguni steers reared on natural pasture than those that entirely relied on rangeland. Mapiye *et al*. (2011) indicated that the greater performance generally displayed by ruminants fed dried browse legume leaves is partially credited to a high concentration of CP, moderate digestibility and desirable effects of condensed tannins. Regarding the use of browse species pods, Marius (2016) supplemented Namibian indigenous goats on natural pasture with *V. erioloba* and *D. cinerea* pods, resulting in improved growth and milk yield. Another study by Yayneshet *et al*. (2008) also observed positive growth of up to 21.7 g/day, improved dressing percentage and high muscle (lean) weight on smallholder goats in Ethiopia supplemented with *D. cinerea* and *V. etbaica* pods during the dry season. Gebru *et al*. (2018) observed positive but similar growth rates, dressing percentage and rib eye areas on Abergelle rams supplemented with pods of *V. tortilis, Faidherbia albida* and *D. cinerea* at the rate of 10 g kg^{-1} body weight daily.

2.12 **Methane production from ruminants fed browse**

Emission of methane (CH_4), irrespective of the sources is considered as one of the most important global environmental issues, contributing considerably to greenhouse gasses (IPCC, 2001). Ruminants are also a major contributor to the production of enteric methane (CH_4) in the world, during the normal anaerobic microbial fermentation of feed in the rumen (Brouček, 2015; Macome *et al*., 2017). Goel and Makkar (2012) described the ruminal methane (CH_4) production as a by-product of the microbial digestive process in the rumen and represents a loss of 2–12% of the feed energy. This is especially the case when feeding highly fibrous diets which are prevalent in the tropics.

Broucek (2014) reported that many factors influence ruminant CH_4 production, including level of intake, type and quality of feeds, energy consumption, animal size, growth rate, level of production, genetics and environmental temperature. Several studies have reported CH_4 emissions from dairy cattle feeds and feed ingredients using *in vitro* techniques (Macome *et al*., 2017). For example Broucek (2014) reported that methane emissions in dairy cows represent values from 151 to 497 g·day^{-1}, while the average CH_4 emissions are from 161 g·day^{-1} to 323 g·day^{-1} in beef cattle. Therefore, it has become important to reduce CH_4 emissions associated with the production of milk and meat, to reduce their ecological footprint.

Considerable efforts have been made to explore the possibility of assessing methane production from animals using an *in vitro* technique (Melesse *et al*., 2013, 2019). Other studies investigated the possibility to assess CH_4 emissions from rumen fermentation using the *in vivo* method and were summarised by Goel and Makkar (2012). However, *in vivo* experiments were reported to be very expensive, laborious and time consuming. Therefore, Macome *et al*. (2017) suggested that if *in vivo* CH_4 emission could be estimated using equations based on *in vitro* data, more rapid gains in the knowledge and understanding of factors influencing CH_4 emission from ruminants could be achieved.

Theart *et al*. (2015) and Abdulrazak *et al*. (2000) reported that inclusion of browse species containing plant secondary metabolites, such as tannins and saponins in diets of ruminants, seems promising as a nutritional strategy to reduce CH_4 emissions from ruminants. The condensed tannins decrease methane more through reduction in fibre digestion (indirect effect), while hydrolysable tannins appear to act more through inhibition of the growth and/or activity of methanogens and/or hydrogen-producing microbes (direct effect) (Goel and Makkar, 2012). Hydrolysable and condensed tannins and their extracts have been shown to decrease methane (CH_4) production under both *in vivo* and *in vitro* conditions. For example, condensed tannins from some legume trees have been reported to reduce methane production (g/kg DMI) by about 15% in sheep and by a similar amount in dairy cows. Unlike tannins, a limited number of *in vivo* studies have been conducted with saponins, using Yucca as source of saponins. However, for *in vitro* methods, the saponin-containing plants did not produce substantial reduction in methane production but showed the potential to partition higher proportion of the substrate to microbial mass production (Goel and Makkar, 2012).

Recent research work had also indicated that there are several methane- mitigating feed additives being developed and tested *in vitro* and on commercial farms around the world. These include: red seaweed, enzyme inhibitor (3-Nitrooxypropanol), nitrate, essential oil blends, antibiotic rumen modifiers and Biochar (Hegarty *et al*., 2021; Honan *et al*., 2021). While each additive works differently, they all essentially work by preventing methane formation from ruminants and their effectiveness can range from 10 to 60% (Hegarty *et al*., 2021).

2.13 **Conclusion**

The chemical composition of the selected browse species have demonstrated their nutritional potential, generally as feed resources and in particular as protein sources or supplements especially during dry seasons when the quality of basal forages is low or during drought. Due to variability in preference by different animal species, the presence of a variety of browse species may also be important during periods of feed scarcity to improve available nutrients to animals. Future research should aim at exploring alternative means to sustainably harvest and utilize encroacher browse plants that are available in abundance. There is, however, a need to understand their full nutritional value, anti-nutritional factors and even the fermentation chemistry of different browse species to diversify into other browse products such as silage.

CHAPTER 3

Nutritional value of four Namibian encroacher bush species as potential fodder for ruminants

3.1 Abstract

Encroacher bushes have the potential to be used as animal feed resources because they can provide considerable amount of biomass from leaves and small twigs as well as pods. The objective of this study was to evaluate the nutritive value of four Namibian encroacher bush species in different seasons. Leaves and twigs (< 20 mm stem diameter) were harvested during the late dry (August), early rainy (December) and late rainy (May) seasons, shade-dried and analysed for chemical composition. The chemical composition was influenced ($P < 0.05$) by species x season interaction. Crude protein (CP) ranged from 46.0 g/kg DM in *T. sericea* to 111.7 g/kg DM in *S. mellifera*. The NDF and ADF contents for *D. cinerea* and *S. mellifera* decreased ($P < 0.05$) from late dry to early and late rainy season, while for *R. trichotomum* and *T. sericea* increased. The NDF and ADF concentration ranged from 594.2 to 734.3 g/kg DM and 463.21 to 578.6 g/kg DM, respectively; with *R. trichotomum* having the lowest contents while *D. cinerea* had the highest. The soluble true protein was relatively low (less than 10 g/100g CP) in all bush species, except for *S. mellifera*. Acid detergent insoluble protein represented a large proportion for *T. sericea* in both seasons, being 42.2 g/100g CP for the late dry season and 52.4 g/100g CP for the early rainy season. The concentration of polyphenols (Total phenols, total tannins and hydrolysable tannins) increased ($P < 0.001$) from the late dry to the early rainy season, except for condensed tannins. The concentration of condensed tannins ranged from 1.86 to 24.8 g/kg DM, with *T. sericea* containing the highest ($P < 0.05$) concentration while *R. trichotomum* had the lowest. Mineral concentrations was influenced ($P < 0.05$) by species, seasons and species x season interactions. Both macro and micro minerals showed an increased peak in early rainy season and a decrease in late rainy and or late dry seasons, except for the iron content which was exceptionally high ($P < 0.001$) in the late dry season compared to the rainy season. All species were relatively low in phosphorus, sodium, magnesium and zinc, moderate in calcium and copper and

high in iron and manganese. The non-essential amino acids, constituted about 51 - 60% of total amino acids with proline, aspartic acid and glutamic acid being dominant, while arginine, leucine and valine were dominant among the essential amino acids. Linoleic acid (C18:2n-6) was the main fatty acid present in all four species, ranging from 23.5 g/100g FA for *S. mellifera* to 37.1 g/100g FA for *D. cinerea*, followed by palmitic acid (C16:0), oleic acid (C18:1c9), α-linolenic acid (C18:3n-3) and stearic acid (C18:0). In conclusion, based on the chemical composition of the four species investigated in this study, they could generally be considered of intermediate nutritional quality.

3.2 Introduction

Livestock production in Namibia, particularly for cattle, goats and sheep depends highly on the natural rangelands comprising of grasses and browse plant species, for feed resources. However, according to Shikangalah and Mapani (2020), approximately 45 million hectares of Namibian farmlands are being progressively taken over by massive growth of woody plants and/or bush encroachment. The encroacher bushes nonetheless have potential for use as animal feed. Browse plants provide considerable amount of biomass from leaves and small twigs of less than 25 mm, as well as pods and maintain their nutrients into the dry season when other feed resources are depleted (Moleele, 1998; Salem, 2005; Quansah and Makkar, 2012).

Senegalia mellifera, *D. cinerea*, *T. sericea* and *R. trichotomum* are classified as dominant encroaching bush species in Namibia (de Klerk, 2004). The nutritional content of some browse plants or bushes such as *Acacia* species were intensively investigated (Abdulrazak *et al*., 2000; Dube *et al*., 2001; Rubanza *et al*., 2005, 2003; Aganga and Mesho, 2008; Mokoboki *et al*., 2019). However, there has been limited research work on the nutritive value of *D. cinerea*, *T. sericea (*Kasale, 2013; Marius, 2016; Naumann *et al*., 2017; Honsbein *et al*., 2017) and *R. trichotomum* (Marius, 2016 and Honsbein *et al*., 2017). Most of the published research on bush species has, however, focused on the browse pods and leaves.

It is also generally known that most browse species contain potentially toxic or digestibility-reducing secondary compounds also known as anti-nutritional factors

(ANFs) such as phenols and tannins (Makkar, 2003). These compounds strongly influence their utilization, especially when consumed in high quantities by interfering with digestion and metabolism (Cooper *et al*., 1988; Bensalem *et al*., 2004; Makkar, 2010). Therefore, knowledge on the concentration of ANFs is important where these bush species are considered as potential feed resources for ruminants.

In addition, Mahesh *et al*. (2017) emphasized that a wide variation in the solubility of dietary protein among various feedstuffs including browse species also exists. It has been suggested that information is needed on the protein fractions that are degraded in the rumen and may be converted to microbial protein, and undegradable or by-pass protein which is more or less digestible in the intestine (National Research Council, 2001; Mahesh *et al*., 2017). Other research gaps such as exploring their amino acids and fatty acid profiles exist.

Due to the encroaching nature of bushes, the browse materials become inaccessible to livestock, thus there is a need to explore other utilization strategies in order to realize their full potential as fodder. Sanon *et al*. (2007) emphasised that cutting bushes to feed livestock is imperative because it allows them to utilise the most edible parts that are not directly accessible. Research attention has been diverted from the removal of encroacher bushes as weeds to their utilization as animal feed (Mapiye *et al*., 2011). This has encouraged the widespread adoption of feeding strategies based on encroacher bushes and it has been gaining momentum in Namibia in recent years (Honsbein *et al*., 2017), both as a drought relief strategy and also for commercial purposes. Therefore, the objective of this study was to evaluate the fodder potential of *S. mellifera, D. cinerea*, *T. sericea* and *R. trichotomum* based on their chemical composition, polyphenol contents, protein fractions, minerals, amino acids and fatty acid composition in different seasons of the year.

3.3 **Materials and Methods**

3.3.1 **Selection of the bush species and study areas**

The encroacher bush map shown in Figure 3.1 was used as a guide for species selection. Four (4) encroacher bush species around Namibia were considered in this study. The criteria for selection was based on the species with the highest recorded densities, exceeding 5000 plants/hectare, according to Smit *et al*. (2015), which is regarded as impenetrable by livestock. This implies that in relation to other species, they have more negative impacts on livestock production and wildlife habitats, hence adversely affecting the country's economy.

Study areas for sample collection were selected based on the area where the encroacher species are predominantly found (Figure 3.1); it should be noted that we did not determine the bush density in this study. Hence, within the context of this study, three (3) study areas representing respective dominant encroacher species were selected, namely: John Alphons Pandeni Research Station (JAP) previously known as Uitkomst Research Station for *Senegalia mellifera* and *Dichrostachys cinerea;* Sandveld Research Station (SAN) for *Terminalia sericea* and Kalahari Research Station (KAL) for *Rhigozum trichotomum*.

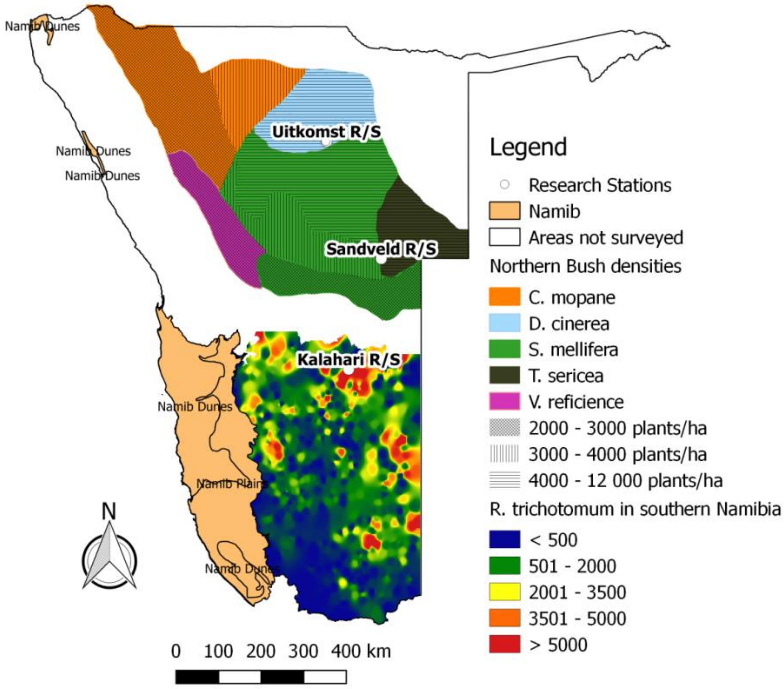

Figure 3.1 Occurrence of dominant encroacher species in Namibia and location of 3 selected study areas (Source: de Klerk, 2004)

John Alphons Pandeni Research Station (JAP) is located 20 km south-west of Grootfontein, on the Grootfontein-Otavi road, in Otjozondjupa region. The size of the farm is 6559.4 ha. It falls under KAL3 of the Agro-ecological zones of Namibia. The vegetation type is mostly mountain savannah and Kartz veld, but the southern part of the farm is Thornbush Savannah. The average annual rainfall of the farm is 517 mm.

Sandveld Research Station (SAN) is located approximately 60 km northeast of Gobabis in the Omaheke region of Namibia. The size of the farm is 8366 ha and falls under the Kal3-4 of the Agro-ecological zones of Namibia and its vegetation type is a Camelthorn savannah. The average annual rainfall of the farm is 392 mm.

Kalahari Research Station (KAL) is located approximately 28 km north-east of Stampriet on the Leonardville road in the Hardap region of Namibia. The size of the farm is 10 215 ha and falls under the Mixed tree and thorn Savannah type of vegetation. The average annual rainfall of the farm is 184 mm.

The annual rainfall distribution of the year preceding the study 2017/2018 and for the study seasons 2018/2019 are presented in Figure 3.2 and Figure 3.3, respectively.
The rainfall records data were obtained from the SASSCAL weather net website, (http://www.sasscalweathernet.org/index.php?MIsoCode=NA) and supplemented with manual rainfall data records from respective research stations.

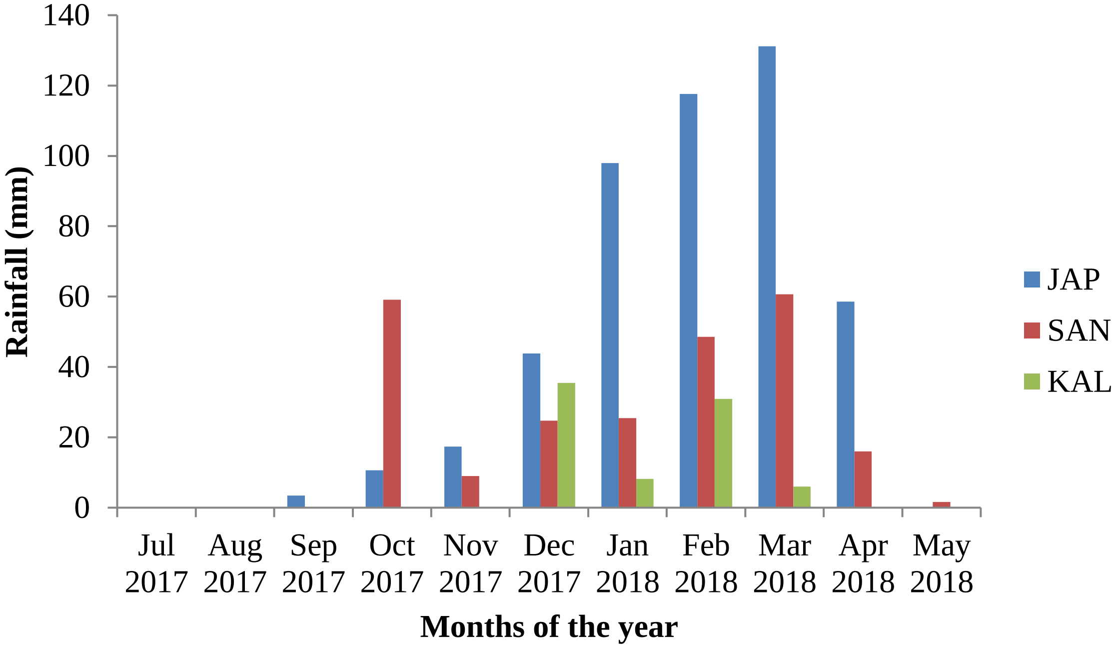

Figure 3.2 Rainfall distribution of the year 2017/2018 preceding the study in the 3 study

areas: John Alphons Pandeni Research Station (JAP), Sandveld Research Station (SAN) and Kalahari Research Station (KAL)

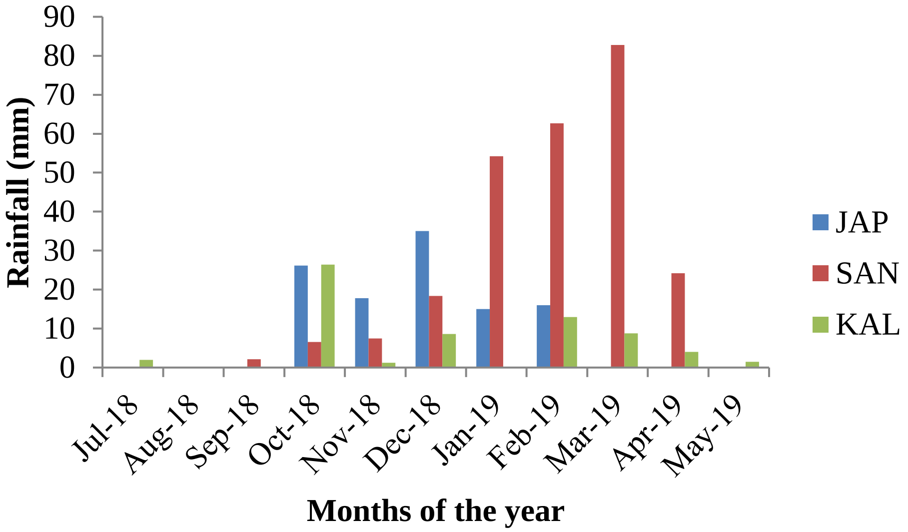

Figure 3.3 The rainfall distribution for the study year 2018 in the 3 study areas: John Alphons Pandeni Research Station (JAP), Sandveld Research Station (SAN) and Kalahari Research Station (KAL)

3.3.2 Sampling methods

The sampling design used combined the criterion of purposeful sampling and a stratified two-stage probability sampling in order to select the sample collection sites and individual bushes sampled. Ten (10) sites were randomly selected in each study area using satellite imagery as shown in Figure 3.4. The geographical coordinates were obtained and entered in the GPS device to locate the sites on the ground. At JAP where two species were selected, ten sites were initially selected with satellite imagery with the assumption that both species would be present at all sites. However, on the ground the 2 species were encroaching different areas on the farm. Therefore, the number of sites were re-adjusted to 20 with 10 sites for *S. mellifera* and the other 10 sites for *D. cinerea*. Ten (10) bushes were sampled at each site for each species, and all the sampled materials from that site were pooled together to form one sample. Collected samples from respective sites were regarded as replicates for the selected encroaching bush species.

The chemical compositions were analyzed based on the 10 replicate samples for each species described above. However, it was not possible to do the protein fractions, polyphenols, amino acids and fatty acids analysis from the 10 replicate samples of each species due to cost implications, the capacity of the some of the equipment used and complexity of the processes involved in the analysis. In that case, only 3 of the 10 replicates for each species were randomly selected for analysis.

Figure 3.4 Sample collection sites within the 3 selected study areas. From left: Kalahari Research Station (Kal1-Kal10), John Alphons Pandeni Rese

(JAP1-P10), Sandveld Research Station (San1-San10)

3.3.3 Sample collection and preparation for laboratory analyses

Samples of leaves and twigs of 20 mm or less in stem diameter (to mimic the size used by for bush feed production) from the selected encroacher bush species were harvested with a pruning scissor. Samples were collected in August 2018 (late dry season), December 2018 (early rainy season) and May 2019 (late rainy season). Samples were air dried under the shade for 5 days and ground in a hammer mill to pass through a 1 mm screen. Ground samples were stored in tightly closed plastic bottles, for subsequent laboratory analysis.

3.3.4 Chemical analysis

The dry matter (DM) content of bush species samples was determined by drying the samples in a forced draught oven at 100 °C for 24 h (AOAC, 2000). Ash was determined by incineration in a muffle furnace at 550 °C for 6 h (AOAC, 2000). The crude protein (CP) method no. 978.04 (AOAC, 2005) was used to determine the total nitrogen content and CP was estimated by multiplying percentage of N content by a factor 6.25. Ether extract (EE) was determined using the AOAC method 920.39 (AOAC, 2000). Ash-free neutral detergent fibre (NDFom) and Ash-free acid detergent fibre (ADFom) were determined following the procedures of Mertens *et al*. (2002) with NDFom assayed without use of an alpha-amylase, but with sodium sulfite. Ash-free Acid detergent lignin (ADLom) was determined by placing dried ADF samples in 72% sulphuric acid (H_2SO_4) (Mertens, 2015). Hemicellulose content was calculated as difference between NDFom and ADFom, while cellulose as a difference between ADFom and ADLom (Rinne *et al*., 1997). The concentration of macro and micro elements was analysed using the inductively coupled plasma-atomic emission spectrometry (ICP-AES) (icap 6000 series) methods (AOAC, 2007), except for phosphorus (P) content which was determined using DR6000^{TM} UV VIS spectrophotometer (Hach, UK) (AOAC, 2005). Organic matter (OM) was calculated by subtracting the ash content from dry matter.

3.3.5 Measurement of anti-nutritional factors: total phenols and tannins

Determination of total phenol (TP), total tannins (TT) and condensed tannins (CT) was based on methods as described by Makkar (2003). The TP and TT were determined according to modified Folin-Colicalteu method by using polyvinyl-polypyrrolidone

(PVPP) to separate tannin phenols from non-tannin phenols. The TP and TT were both calibrated against tannic acid as a standard and values expressed as tannic acid equivalents. The CT were determined using butanol-HCl-iron method and recorded absorbance at 550 nm (Singh *et al*., 2005) using DR6000^{TM} UV VIS spectrophotometer (Hach, UK). The CT values obtained were expressed as leucocyanadine equivalents as condensed tannins (% in dry matter), calculated by the formula: (A 550 nm x 78.26 x Dilution factor)/(% dry matter). The hydrolysable tannins (HT) was estimated as a difference between TT and CT (Singh *et al*., 2005).

3.3.6 **Protein fractionation**

The fractionation of nitrogen as non-protein nitrogen (NPN), soluble protein and nitrogen, neutral-detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were measured according to the methods described by Licitra *et al*. (1996). The NPN is soluble in buffer and tungstic acid; B1 fraction (true soluble protein) is soluble in borate-phosphate buffer and precipitated by tungstic acid; Fraction B2 (neutral detergent soluble protein, NDSP) is insoluble in buffer solution but soluble in neutral detergent, fraction B3 (acid detergent soluble protein, ADSP) is soluble in acid detergent but insoluble in neutral detergent, fraction C (not fermented and unavailable to the animal) is insoluble in acid detergent. The CP fractions were then calculated as non-protein nitrogen (NPN, A Fraction) and as true proteins (B1, B2, B3 and C fractions) based on their rumen degradability rate (Acar, 2018; Acar *et al*., 2019). The A+B1 fractions generate total soluble protein (SolP).

3.3.7 **Amino acid analysis**

Quantitative determination of free and bound proteinogenic amino acids in bush samples was carried out by ultra-performance liquid chromatography (UPLC) at the University of Helsinki, Department of Agricultural Sciences, Finland. The method is based on the extraction of free amino acids from plant materials, acid hydrolysis, and subsequent analysis of hydrolytes by a Waters Acquity Ultra Performance Liquid Chromatography (Milford, A, USA) with a Waters binary system, following methodology by Meussen *et al*. (2014). The derivatives were separated using C18 column (Acquity UPLC BEH C18 100 mm x 2.1 mm, 1.7 µm particle size).

3.3.8 **Fatty acid analysis**

Fatty acid methyl esters (FAME) of air-dried and milled bush samples were prepared in a one-step extraction transesterification procedure using chloroform according to Sukhija and Palmquist, (1988), with the exception that methanolic sulphuric acid (2% v/v) was used as the methylating reagent. Tridecanoic acid (13:0) methyl ester as an internal standard, was also added to the samples prior to fat extraction and methylation. The FAME were separated and quantified by gas-liquid chromatography-mass spectrometry analysis using a Shimadzu GCMS-QP2010 Ultra spectrometer (Shimadzu Europa GmbH), at the University of Helsinki, Department of Agricultural Sciences, Finland.

3.3.9 **Statistical analysis**

The experimental design was a factorial arrangement of treatments either with 4 species x 3 seasons (for chemical composition and anti-nutritional factors) or 4 species x 2 seasons (for protein fractions, amino acids and fatty acids). The third season data for protein fractions, amino acids and fatty acids was not available because the equipment and consumables needed to analyse them were not available in Namibia, whereas the first two seasons' analyses were done in Finland during the short term research visit. Therefore, data were analysed based on 4 x 2 or 4 x 3 nested-factorial treatment design within a completely randomised design, and subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS Institute Inc (2009). Data transformation was carried out on ADLom and ash using logarithms to ensure data had an approximate normal distribution before analysis. The main effects were bush species and season. Sampling site nested within species was treated as a random effect. The following statistical model was used:

$Y_{ijkm} = \mu + F_i + S_j + T_{k(j)} + FS_{ij} + e_{ik(j)} + e_{ikm.}$

Where:

Y_{ijkm} = dependent variable (DM, OM, ash, CP, etc.)

μ = overall mean;

F_i = effect of browse species (i= 1, 2, 3, 4);

S_j = effect of season (j = 1, 2, 3);

$T_{k(j)}$ = effect of sampling site (k = 1, 2, 3…10) nested within species (i = 1, 2, 3, 4);

$(FS)_{ij}$ = interaction effect of browse species and season;

$e_{ik(j)}$ = random component explaining variation among experimental units (EU) on the same treatment;

e_{ijkm} = random component explaining variation within EU, among subsamples of the same EU.

For the statistical analysis, seasons were defined as late dry season [LDS] (August – November); early rainy season [ERS] (December – March); and late rainy season [LRS] (April - July). Means were separated using the Tukey' Studentised range test. Differences among means with $P < 0.05$ were considered significant. In addition, differences among means with $0.05 \leq P \leq 0.10$ were regarded as representing tendencies.

3.4 **Results**

3.4.1 **Chemical composition**

The chemical composition of the four encroacher bushes species were influenced ($P < 0.0001$) by species, site nested with species, season and the interaction between season and species (Table 3.1). The dry matter (DM) content of all species increased ($P < 0.05$) from late dry season to early rainy season, then dropped ($P < 0.0001$) in the late rain season. During the late dry season, the DM content of all species were similar, except that of *T. sericea* which was lower ($P < 0.0001$) than others. The OM content was highest ($P < 0.05$) during the early rainy season; *T. sericea* maintained the lowest ($P < 0.0001$) OM content in all seasons.

The ash content of all species decreased ($P < 0.05$) progressively from late dry to early and late rainy seasons, with exception of *D. cinerea*, which increased ($P < 0.05$). Ether extract (EE) was similar ($P > 0.05$) across seasons for *D. cinerea*; decreased ($P < 0.05$) for *R. trichotomum* during the late rainy season; increased for *S. mellifera* from the late dry season to the early rainy season and then decreased ($P < 0.05$) during the late rainy season. For *T. sericea* the EE increased ($P < 0.05$) progressively from the late dry season right through to the late rainy season.

The crude protein (CP) content for *D. cinerea* and *S. mellifera* increased ($P < 0.0001$) from dry season to early rainy season then dropped again in the late rainy season, while *T. sericea* had similar CP content in late dry and early rainy seasons, then only increased ($P < 0.0001$) in the late rainy season. *R. Trichotomum* declined ($P < 0.05$) in CP during the late rainy season. *S. mellifera* recorded the highest CP, with 111.8 g/kg DM during the early rainy season and 97.4 g/kg DM in the late rainy season. *T. sericea* recorded the lowest ($P < 0.001$) CP across all seasons.

The content of neutral detergent fibre (NDFom) for *D. cinerea* and *S. mellifera* decreased ($P < 0.0001$) from late dry to early rainy season then increased again in the late rainy season. The NDFom for *T. Sericea* decreased ($P < 0.05$) during the late rainy season; whereas a progressive increase ($P < 0.001$) in NDFom and ADFom for *R. trichotomum* from late dry to early rain and late rainy seasons were observed. For ADFom, *S. mellifera* did not show ($P > 0.05$) seasonal effects. *Terminalia sericea* had lower content of NDFom in the late rainy season ($P > 0.05$) compared to the other two seasons. The ADFom content of *T. sericea* decreased ($P < 0.05$) from late dry to early rain season, then increased again in the late rainy season. *Dichrostachys cinerea* had higher ($P < 0.05$) NDFom and ADFom than other species. The species exhibited different ADLom contents in response to seasonal changes, with an increasing trend from dry to early rainy and late rainy seasons. *D. cinerea* had higher NDFom (734.3 g/kg DM), ADFom (579.6 g/kg DM), ADLom (184.4 g/kg DM), hemicellulose (155.7 g/kg DM) and cellulose (415.1 g/kg DM) than other species in all the 3 seasons.

Table 3.1 The chemical compositions (g/kg DM) of four bush encroacher species in different seasons

		Parameters									
Bush species	**Season**	**DM**	**OM**	**Ash**	**EE**	**CP**	**NDFom**	**ADFom**	**ADLom**	**Hem**	**Cel**
D. cinerea	Late DS	939.6^{g}	907.7^{b}	31.5^{h}	9.2^{f}	66.5^{f}	734.3^{a}	578.6^{a}	163.4^{de}	155.7^{a}	415.2^{a}
	Early RS	954.3^{b}	916.5^{a}	37.4^{f}	9.7^{ef}	88.8^{c}	689.3^{b}	569.1^{a}	184.4^{c}	120.1^{d}	382.9^{b}
	Late RS	945.6^{d}	905.9^{b}	39.3^{ef}	9.7^{ef}	60.4^{g}	734.0^{a}	579.9^{a}	222.8^{a}	154.0^{a}	369.3^{b}
R. trichotomum	Late DS	940.5^{fg}	900.3^{c}	40.2^{e}	19.6^{ab}	74.9^{e}	594.3^{f}	463.2^{e}	137.5^{g}	131.1^{c}	325.7^{d}
	Early RS	952.7^{c}	917.3^{a}	35.3^{g}	20.5^{a}	74.3^{e}	641.6^{d}	505.9^{d}	153.1^{ef}	135.6^{bc}	352.9^{c}
	Late RS	951.6^{c}	916.9^{a}	34.5^{g}	10.9^{e}	69.7^{f}	667.0^{c}	540.7^{b}	188.2^{c}	124.5^{cd}	368.6^{b}
S. mellifera	Late DS	934.0^{fg}	883.9^{e}	55.5^{c}	12.8^{d}	82.1^{d}	667.7^{c}	518.8^{cd}	144.1^{fg}	148.9^{ab}	374.8^{b}
	Early RS	957.0^{a}	906.1^{b}	50.5^{d}	19.1^{b}	111.8^{a}	647.4^{d}	511.6^{c}	144.1^{fg}	135.8^{bc}	367.5^{bc}
	Late RS	943.5^{e}	891.1^{d}	52.2^{d}	15.1^{c}	97.5^{b}	675.2^{bc}	524.0^{c}	156.2^{ef}	151.2^{a}	358.8^{c}
T. sericea	Late DS	924.0^{h}	856.9^{f}	66.7^{a}	7.2^{g}	46.9^{i}	617.6^{e}	504.3^{d}	148.7^{ef}	113.3^{de}	355.6^{c}
	Early RS	952.5^{c}	890.5^{d}	61.3^{b}	8.6^{f}	47.9^{i}	620.2^{e}	522.2^{c}	168.0^{d}	98.0^{e}	354.3^{c}
	Late RS	941.5^{f}	884.7^{e}	56.4^{c}	14.9^{c}	52.9^{h}	600.7^{f}	506.0^{d}	208.6^{b}	100.9^{e}	302.7^{e}
SEM		0.6	1.0	0.9	0.5	1.4	5.6	5.5	3.4	5.6	5.6

P-value	Species	0.3461	0.0381	0.0121	0.0219	0.0699	0.1075	0.0496	0.0599	0.1405	0.1299
	Season	0.0152	0.0291	0.4599	0.1944	0.1837	0.8574	0.4355	0.0625	0.1683	0.7963
	Species x Season	<0.0001	<0.0001	<0.0001	< 0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.002	<0.0001
R-Square		0.936	0.950	0.938	0.851	0.926	0.816	0.723	0.77	0.517	0.72

Legend: DM = dry matter, OM = Organic matter; EE = Ether extract; CP = Crude protein; NDFom = ash-free neutral detergent fiber; ADFom = ash-free acid detergent fiber; ADLom = ash-free acid detergent lignin; Hem = Hemicellulose; Cel = Cellulose; SEM = Standard error of means

$^{a-i}$Means with different superscripts within a column differ significantly **(**$P < 0.05$**)**

3.4.2 **Protein fractions**

The effects of interactions of season and species on the different protein fractions are shown in Table 3.2. The species by season interactions affected ($P < 0.05$) NPN, NDSP, ADSP, ADIP, but not ($P > 0.05$) STP. The NPN for *T. sericea* and *D. cinerea*, decreased from dry season to rainy season, for *R. trichotomum* it remained similar across the seasons, while for *S. mellifera* it increased from dry to rainy season.

Among the four encroacher species, *S. mellifera* had the highest ($P < 0.05$) soluble true protein (STP) even though within each species the levels of STP were similar across the seasons. Except for *R. trichotomum*, the neutral detergent soluble protein (NDSP) for the other three species decreased from dry to rainy season, with a significant ($P < 0.05$) decrease for *D. cinerea.* The acid detergent soluble protein (ADSP) for *D. cinerea* and *S. mellifera* increased ($P < 0.05$) during the early rainy season; for *R. trichotomum* it remained similar over the two seasons; while it decreased ($P < 0.05$) during the early rainy season for *T. sericea*. *Terminalia sericea* had higher ($P < 0.05$) concentrations of ADIP (C) than other species. The ADIP for *D. cinerea* and *S. mellifera* was similar ($P > 0.05$) for the two seasons; ADIP for *R. trichotomum* decreased ($P < 0.05$) during the early rainy season, but increased ($P < 0.05$) for *T. sericea* during the early rainy season.

Table 3.2 The protein fractions (g/100g CP) of four encroacher bush species in different seasons

		Protein fractions				
Bush species	**Season**	**NPN (A)**	**STP (B1)**	**NDSP (B2)**	**ADSP (B3)**	**ADIP (C)**
D. cinerea	Late DS	23.7^{e}	6.8^{b}	27.7^{a}	10.9^{bc}	30.9^{cde}
	Early RS	17.7^{f}	9.9^{b}	20.8^{b}	21.0^{a}	30.7^{cde}
R. trichotomum	Late DS	43.1^{a}	8.4^{b}	10.8^{c}	9.1^{bc}	28.5^{ec}
	Early RS	44.4^{a}	6.5^{b}	19.5^{b}	6.0^{c}	23.7^{f}
S. mellifera	Late DS	34.3^{c}	22.4^{a}	9.2^{cd}	0.6^{d}	33.5^{cd}
	Early RS	35.9^{b}	18.5^{a}	5.0^{d}	8.1^{bc}	32.5^{cd}
T. sericea	Late DS	28.2^{d}	7.4^{b}	6.7^{cd}	15.5^{a}	42.2^{b}
	Early RS	23.9^{e}	8.1^{b}	8.5^{cd}	7.0^{bc}	52.5^{a}
SEM		0.4	1.3	1.2	1.5	0.9
P-value	Species	0.0108	0.0219	0.1016	0.4238	0.0598
	Season	0.3998	0.7594	0.9691	0.7606	0.761
	Species xSeason	0.001	0.183	0.009	0.009	0.004
R-square		1.0	0.97	0.99	0.97	0.99

NPN = Non-protein nitrogen; STP = soluble true protein; NDSP = Neutral detergent soluble protein, ADSP = acid detergent soluble protein; ADIP = acid detergent insoluble protein. SEM = Standard error of mean

$^{a - f}$Means with different superscripts within a column differ significantly ($P < 0.05$).

3.4.3 **Anti-nutritional factors: total phenols and tannins**

Total phenols and tannins (g/kg DM) of the four encroacher bush species across seasons are shown in Table 3.3. Species x season interaction influenced ($P < 0.0001$) all the ANFs measured in this study. The concentration of TP, TT and HT increased ($P < 0.0001$) from late dry to late rainy season for *D. cinerea* and *S. mellifera,* but for *R. trichotomum,* the concentrations only increased from late dry to early rainy and remained similar in the late rainy season. On the contrary, the TP, TT and HT for *T. sericea* increased ($P < 0.05$) during the early rainy season, but declined during the late rainy season.

In the late dry season, *S. mellifera* had the lowest ($P < 0.05$) levels of TP, TT, CT and HT compared to other species. *Senegalia mellifera* still maintained the lowest ($P < 0.0001$) concentrations for TP, TT and HT in the early and late rainy seasons. *Rhigozum trichotomum* and *S. mellifera* maintained the lowest levels of CT across the 3 seasons. The concentration of CT for *D. cinerea* progressively increased ($P < 0.0001$) from late dry to late rainy season, while that of *R. trichotomum* and *T. sericea* decreased ($P < 0.05$). Comparison of the early and late rainy seasons for all the four species, shows that *T. sericea* had the highest concentrations of all the ANFs.

Table 3.3 The concentration of phenols and tannins (g/kg DM) of four encroacher bush species in three different seasons

Browse species	Season	Components			
		TP	**TT**	**CT**	**HT**
D. cinerea	Late DS	17.5^{f}	8.9^{g}	4.5^{e}	4.47^{i}
	Early RS	53.2^{d}	50.5^{d}	14.7^{d}	35.87^{def}
	Late RS	75.7^{c}	72.9^{c}	17.0^{c}	55.84^{c}
R. trichotomum	Late DS	45.4^{d}	19.7^{f}	3.1^{ef}	16.58^{gh}
	Early RS	65.8^{c}	42.9^{de}	1.9^{fg}	41.01^{de}
	Late RS	67.1^{c}	43.4^{d}	0.9^{fg}	42.46^{de}
S. mellifera	Late DS	10.8^{f}	4.0^{g}	2.4^{efg}	1.55^{hi}
	Early RS	28.0^{e}	20.1^{f}	2.3^{fg}	17.78^{g}
	Late RS	42.6^{d}	31.2^{e}	1.93^{fg}	29.23^{ef}
T. sericea	Late DS	43.4^{d}	33.1^{e}	24.82^{a}	8.25^{ghi}
	Early RS	207.7^{a}	194.6^{a}	23.72^{ab}	170.84^{a}
	Late RS	139.3^{b}	135.5^{b}	22.41^{b}	113.08^{b}
SEM		3.3586	3.2345	0.6590	3.6221
P-value	Species	<0.0001	<0.0001	<0.0001	<0.0001
	Season	<0.0001	<0.0001	0.0049	<0.0001
	Species x Season	<0.0001	<0.0001	<0.0001	<0.0001
R-square		1.0	1.0	1.0	1.0

TP = Total phenolics; TT = Total tannins; CT = Condensed tannins; HT = Hydrolysable tannins; SEM = Standard error of mean

$^{a-i}$Means with different superscripts within a column differ significantly **(**P < 0.05**)**

3.4.4 Macro and micro minerals

The composition of macro (Ca, P, K, Mg and Na) and micro (Mn, Cu, Fe and Zn) mineral concentrations of the four encroacher bush species in different seasons are presented in Table 3.4. The species x season interaction influenced ($P < 0.05$) the mineral concentrations, except K and Mg which showed a trend ($P < 0.10$). The highest ($P < 0.001$) Ca contents were observed for *T. sericea* in all seasons compared to other species, but decreased as seasons progressed to the early and late rainy seasons. On the other hand, *R. trichotomum* presented no ($P > 0.05$) seasonal change and had the lowest Ca concentrations across all seasons compared to other species; *D. cinerea* and *S. mellifera* only increased ($P < 0.05$) Ca contents in the late rainy and early rainy seasons, respectively.

Terminalia sericea recorded the lowest ($P < 0.001$) concentrations for phosphorus (P) in all seasons compared to other species, while *R. trichotomum* had the highest. *Rhigozum trichotomum* and *S. mellifera* had similar P contents in the late dry season, but the concentrations gradually decreased from the late dry season to the late rainy season. *Dichrostachys cinerea* increased ($P < 0.05$) in P content in the early rainy season.

The potassium (K) and Mg concentration of all species tended to increase ($P = 0.078$) from late dry season to early rainy season; then showed a tendency to drop ($P = 0.083$) from the early rainy to late rainy season. *Senegalia mellifera* decreased ($P < 0.05$) in the Na content from late dry season to early rainy season and late rainy season, whereas an increase ($P < 0.05$) was observed for *D. cinerea*, *R. trichotomum* and *T. sericea* from the late dry to late rainy seasons.

In terms of micro minerals, iron (Fe) was abundantly present in all species, especially in the late dry season compared to the two rainy seasons ($P < 0.0001$); manganese (Mn) was more abundant in *T. sericea* ($P < 0.0001$) compared to other species and for zinc (Zn) in *R. trichotomum*, than in the other three species, across all seasons. Copper (Cu) had the lowest concentrations of all the micro minerals analysed and increased ($P < 0.05$) in the early rainy season, but then dropped ($P < 0.05$) in the late rainy seasons for *D. cinerea*, *R. trichotomum* and *S. mellifera*; for *T. sericea* the Cu concentration decreased ($P < 0.05$) during the early rainy season, but increased ($P < 0.05$) during the late rainy season.

Table 3.4 Macro (g/kg DM) and micro (mg/kg DM) minerals composition of four encroacher bush species collected in different seasons

Bush species	**Season**	**Macro-minerals**				**Micro-minerals**				
		Ca	**P**	**K**	**Mg**	**Na**	**Cu**	**Fe**	**Mn**	**Zn**
D. cinerea										
	Late DS	4.71^{f}	0.60^{c}	3.49^{d}	0.78^{cd}	0.02^{e}	1.49^{de}	182.20^{b}	7.22^{f}	6.95^{e}
	Early RS	5.88^{ef}	0.75^{ab}	4.29^{c}	0.99^{a}	0.06^{bc}	2.13^{cd}	26.80^{gh}	6.69^{f}	9.08^{cd}
	Late RS	7.64^{de}	0.65^{bc}	4.26^{c}	0.85^{bc}	0.06^{bc}	1.63^{d}	44.40^{ef}	5.91^{f}	7.81^{cde}
R. trichotomum	Late DS	5.62^{f}	0.80^{a}	6.47^{b}	0.61^{de}	0.07^{b}	3.01^{b}	101.60^{c}	13.87^{d}	14.55^{a}
	Early RS	3.76^{f}	0.75^{ab}	7.15^{a}	0.63^{cde}	0.07^{b}	3.52^{ab}	48.80^{e}	5.50^{f}	12.72^{ab}
	Late RS	4.55^{f}	0.70^{b}	6.54^{b}	0.56^{e}	0.10^{a}	2.12^{cd}	43.20^{efg}	10.48^{e}	12.36^{b}
S. mellifera	Late DS	11.48^{b}	0.75^{ab}	3.58^{d}	0.91^{ac}	0.12^{a}	1.40^{de}	518.08^{a}	8.04^{ef}	7.31^{de}
	Early RS	9.47^{cd}	0.50^{d}	4.79^{c}	1.00^{a}	0.05^{bcd}	1.85^{d}	24.26^{h}	6.25^{f}	9.52^{c}
	Late RS	10.88^{bc}	0.40^{e}	3.51^{d}	0.88^{ac}	0.03^{de}	0.75^{ef}	44.6^{ef}	5.73^{f}	5.87^{e}
T. sericea	Late DS	15.89^{a}	0.40^{e}	2.76^{e}	0.52^{e}	0.04^{ce}	2.29^{bcd}	83.21^{d}	85.67^{b}	8.44^{cde}
	Early RS	12.02^{b}	0.40^{e}	3.20^{d}	0.89^{ac}	0.07^{b}	0.45^{f}	31.22^{fgh}	80.39^{c}	7.52^{cde}
	Late RS	10.99^{bc}	0.40^{e}	3.22^{d}	0.72^{cd}	0.10^{a}	4.19^{a}	29.08^{fgh}	96.76^{a}	9.41^{cd}
SEM		0.634	0.025	0.186	0.047	0.007	0.2427	5.2131	1.0268	0.6840
P-value	Species	<0.0001	<0.0001	<0.0001	<0.0001	0.0030	0.0003	<0.0001	<0.0001	<0.0001
	Season	0.0226	0.0015	0.0011	0.0023	0.1382	0.5607	<.0001	0.0003	0.2215
	Species x Season	0.0088	0.0004	0.0780	0.0837	<0.0001	<0.0001	<0.0001	0.0002	0.0304
R-square		0.98	0.98	0.99	0.95	0.96	1.0	1.0	1.0	0.95

Ca = Calcium, P = Phosphorus, K = Potassium, Mg = Magnesium, Na = Sodium, Mn = Manganese, Cu = Copper, Fe = Iron, Zn = Zinc; SEM = Standard error of mean; $^{a-h}$Means with different superscripts within a column differ significantly ($P < 0.05$)

3.4.5 **Amino acids**

Fifteen amino acid were analyzed in this study, of which eight were essential amino acids (Arginine, Histidine, Isoleucine, Leucine, Lysine, Phenylalanine, Threonine and Valine), while seven were non-essential amino acids (Serine, Glycine, Aspartic acid, Glutamic acid, Tyrosine, Alanine and Proline). Species by season interactions influenced ($P < 0.005$) amino acid composition except for serine.

The concentration of total essential amino acids increased ($P < 0.05$) from late dry season to early rainy season for all species. The concentration of Histidine increased ($P < 0.05$) for *S. mellifera* and *D. cinerea* from late dry season to early rainy season; it was not altered ($P > 0.05$) for *R. trichotomum* and *T. sericea,* Argine concentration increased ($P < 0.05$) from the late dry season to early rainy season for all species but the magnitude of increase varied among species. Aspartate concentration increased ($P < 0.05$) from the late dry season to the early rainy season for *D. cinerea* and *S. mellifera*, but remained unchanged ($P > 0.05$) for *R. trichotomum* and *T. sericea*. Threonine and lysine increased ($P < 0.05$) from late dry season to early rainy season for *D. cinerea* and *S. mellifera*; remained unchanged for *T. sericea*; and decreased ($P < 0.05$) for *R. trichotomum*. Leucine and Phenyl alanine increased ($P < 0.05$) from the late dry season to early rainy season for *D. cinerea*, *S. mellifera* and *T. sericea*; but remained unchanged ($P > 0.05$) for *R. trichotomum*.

The concentration of Serine was influenced ($P < 0.001$) by species and season showed a trend ($P = 0.059$) to decrease from the late dry season to early rainy season. The least squares means for species were *D. cinerea* 4.84 ± 0.08; *R. trichotomum* 2.81 ± 0.08; *S. mellifera* 5.05 ± 0.08 and *T. sericea* 1.74 ± 0.08. Glycine concentration increased ($P < 0.05$) from the late dry season to the early rainy season for all species except *R. trichotomum*. Glutamic acid and valine concentration increased ($P < 0.05$) from the late dry season to early rainy season for *D. cinerea* and *S. mellifera*; it was unchanged ($P > 0.05$) for *R. trichotomum*; and it decreased ($P < 0.05$) for *T. sericea*. Proline concentration increased ($P < 0.05$) from the late dry season to the early rainy season for *D. cinerea*; showed a trend ($P = 0.072$) to increase for *S. mellifera*; decreased ($P < 0.05$) for *R. trichotomum* and *T. sericea*.

Table 3.5 Essential amino acid composition (g/kg DM) of four encroacher bush species collected in different seasons

		Essential amino acids								
Browse species	**Season**	**His**	**Arg**	**Lys**	**Thr**	**Val**	**Ile**	**Leu**	**Phe**	**Total EAA**
D. cinerea	Late DS	1.79^{c}	3.61^{de}	4.00^{c}	2.33^{d}	3.64^{cd}	2.47^{c}	3.82^{d}	2.55^{de}	24.2
	Early RS	2.06^{b}	4.30^{c}	4.56^{b}	2.88^{b}	4.20^{b}	2.95^{b}	5.12^{b}	3.56^{b}	29.7
R. trichotomum	Late DS	1.35^{d}	5.14^{b}	2.53^{d}	2.62^{c}	3.64^{cd}	2.55^{c}	4.16^{c}	2.40^{ef}	24.4
	Early RS	1.29^{d}	7.79^{a}	2.12^{e}	2.35^{d}	3.46^{cde}	2.47^{c}	4.16^{c}	2.46^{def}	26.1
S. mellifera	Late DS	1.74^{c}	3.50^{def}	4.42^{b}	2.77^{bc}	4.18^{b}	2.86^{b}	4.11^{c}	3.07^{c}	26.7
	Early RS	2.42^{a}	4.56^{c}	5.19^{a}	3.22^{a}	4.55^{a}	3.40^{a}	5.47^{a}	4.01^{a}	32.9
T. sericea	Late DS	1.70^{c}	2.36^{f}	2.53^{d}	1.44^{e}	3.23^{de}	1.47^{d}	2.17^{f}	1.52^{h}	16.5
	Early RS	1.78^{c}	2.96^{ef}	2.45^{de}	1.37^{e}	2.63^{f}	1.37^{d}	2.40^{e}	1.63^{g}	16.6
SEM		0.04	0.16	0.11	0.07	0.07	0.04	0.05	0.03	0.44
P-value	Species	0.0003	0.0005	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
	Season	0.0015	0.0003	0.0467	0.0278	0.4172	0.001	<0.0001	<0.0001	0.0004
	Species x Season	0.0042	0.008	0.0144	0.0107	0.0043	0.0012	0.003	<0.0001	0.0061
R-square		0.99	1.0	1.0	0.99	0.99	1.0	1.0	1.0	1.0

His = Histidine; Arg = Arginine; Lys = Lysine; Thr = Threonine; Val = Valine; Ile = Isoleucine; Leu = Leucine; Phe = Phenylalanine; EAA = essential amino acids; SEM = Standard error of mean; $^{a-h}$Means with different superscripts within a column differ significantly ($P < 0.05$)

Table 3.6 Non-essential amino acid composition (g/kg DM) of four encroacher bush species collected in different seasons

		Non-essential amino acids							
Browse species	**Season**	**Ser**	**Gly**	**Asp**	**Glu**	**Tyr**	**Ala**	**Pro**	**NEAA**
D. cinerea	Late DS	4.81^{a}	2.79^{c}	6.17^{de}	6.14^{bcd}	2.62^{d}	2.75^{cd}	7.11^{e}	32.4
	Early RS	4.86^{a}	3.43^{b}	8.52^{b}	7.18^{a}	3.77^{b}	3.51^{b}	7.72^{d}	39.0
R. trichotomum	Late DS	2.95^{b}	2.92^{c}	5.53^{def}	6.18^{bc}	2.08^{e}	2.72^{cd}	16.25^{a}	38.7
	Early RS	2.65^{b}	2.89^{c}	5.44^{ef}	5.94^{bcd}	2.22^{e}	2.61^{bd}	13.66^{b}	35.5
S. mellifera	Late DS	5.12^{a}	3.25^{b}	7.39^{c}	6.39^{bc}	3.15^{c}	3.36^{b}	5.58^{f}	34.3
	Early RS	4.97^{a}	3.79^{a}	14.37^{a}	7.43^{a}	4.44^{a}	3.85^{a}	5.88^{f}	44.8
T. sericea	Late DS	1.81^{c}	2.54^{d}	3.03^{g}	5.68^{cd}	1.42^{f}	1.63^{e}	8.75^{c}	24.9
	Early RS	1.67^{c}	2.85^{c}	2.90^{g}	4.29^{e}	1.52^{f}	1.76^{e}	7.81^{d}	22.8
SEM		0.11	0.05	0.18	0.13	0.04	0.06	0.16	0.62
P-value	Species	< 0.0001	< 0.0001	< 0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001
	Season	0.059	0.0008	<0.0001	0.2809	<0.0001	0.0013	0.0042	0.0025
	Species x Season	0.5793	0.0151	0.0001	0.002	0.0003	0.0048	0.0017	0.0009
R-square		0.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Ser = Serine; Gly = Glycine; Asp = Aspartic acid; Glu = Glutamic acid; Tyr = Tyrosine; Ala = Alanine; Pro = Proline; NEAA = non-essential amino acids;

SEM = Standard error of mean; $^{a - f}$ Means with different superscripts within a column differ significantly **(**$P < 0.05$**)**

3.4.6 **Fatty acids**

The total fatty acids (TFA) and fatty acid (FA) composition of the four encroacher bush species were influenced ($P < 0.05$) by the interaction of species and season (Table 3.7). The concentration of TFA ranged from 1.17 g/kg DM in *T. sericea* to 2.84 g/kg DM in *R. trichotomum* across the two seasons. There was an observed increase ($P < 0.05$) in the TFA content for *S. mellifera* from late dry season to early rainy season; TFA decreased ($P < 0.05$) for *R. trichotomum* from late dry to early rainy season, while TFA of other species remained similar.

In terms of specific fatty acids, the linoleic acid (C18:2*n*-6) was the main fatty acid present in all four species, ranging from 23.4 g/100g FA for *S. mellifera* to 37.1 g/100g FA for *D. cinerea*. The other FA also present in appreciable concentrations were palmitic acid (C16:0), oleic acid (C18:1*c*9) and α-linolenic acid (C18:3*n*-3) which altogether with C18:2*n*-6 represented on an average 80 g/100g FA. The remaining FAs: stearic acid (C18:0), lauric acid C12:0), myristic acid (C14:0), heptadecanoic acid (C17:0), *cis*-vaccenic acid (C18:1c11), arachidic acid (C20:0), behenic acid (C22:0), lignoceric acid (C24:0), cerotic acid (C26:0) and montanic acid (C28:0), represented a lower proportion of the TFA.

The C18:2*n*-6 decreased ($P < 0.0001$) from late dry season to early rainy season in all four species. There was a considerable variation in the other FA concentration of bush species across seasons. The C18:1*c*9 content for *D. cinerea* and *R. trichotomum* decreased ($P < 0.05$) in the early rainy season, while that of *T. sericea* increased ($P < 0.05$) and no change for *S. mellifera* content. *D. cinerea* and *S. mellifera* had lower ($P < 0.05$) concentrations of C18:3*n*-3 in the early rainy season compared to the late dry season; for *T. sericea* there was no change ($P > 0.05$) in C18:3*n*-3 across the two seasons. The C16:0 content for *D. cinerea, R. trichotomum* and montanic acid for *S. mellifera* remained similar ($P > 0.05$) in the two seasons; the C16:0 concentration for *T. sericea* increased ($P < 0.05$) during early rainy season. Lauric acid C12:0) and arachidic acid (C20:0) increased ($P < 0.05$) from late dry to early rainy season for all four species, while montanic acid (C28:0) only increased in *T. sericea*. There was an observed decrease ($P < 0.05$) in the contents of *cis*-vaccenic acid (C18:1c11) for *D. cinerea* and *S. mellifera* from late dry to early rainy season. The highest contents of behenic acid (C22:0) and lignoceric acid (C24:0) of 6.09 and 8.18 g/100g FA respectively, were recorded for *R. trichotomum*, in the early rainy season.

There were additional four fatty acids: myristoleic acid (C14:1c9); palmitoleic acid (C16:1c9); pentadecylic acid (C15:0) and heneicosanoic acid (C21:0); which were only detected in *R. trichotomum*, but not in the other 3 species. However, they were present in minute quantities between 0.20 and 0.60 g/100gFA.

Table 3.7 Total fatty acids (g/kg DM) and Fatty acid composition (g/100g TFA) of four encroacher bush species collected in different seasons

		Components														
Browse species	**Season**	**TFA**	**12:0**	**14:0**	**16:0**	**17:0**	**18:0**	**18:1 *c9***	**18:1 *c11***	**18:*2* *n*6**	**20:0**	**18:*3* *n*3**	**22:0**	**24:0**	**26:0**	**28:0**
D. cinerea	Late DS	2.38^{b}	0.55^{c}	0.54^{d}	21.46^{d}	1.73^{a}	3.79^{cd}	13.83^{c}	0.84^{cd}	37.08^{a}	1.02^{e}	9.74^{c}	2.20^{de}	4.15^{c}	2.70^{b}	0.87^{c}
	Early RS	2.03^{bc}	1.45^{ab}	1.05^{c}	22.61^{cd}	1.03^{bc}	5.87^{b}	9.07^{e}	0.71^{d}	28.42^{c}	2.25^{c}	12.16^{b}	3.51^{c}	6.04^{b}	4.17^{a}	1.58^{c}
R. trichotomum	Late DS	2.84^{a}	0.40^{c}	2.96^{a}	21.94^{cd}	0.67^{bc}	2.95^{d}	13.95^{c}	1.08^{bc}	26.70^{d}	2.80^{b}	11.44^{bc}	4.72^{b}	5.66^{b}	1.78^{c}	1.54^{c}
	Early RS	2.38^{b}	0.90^{b}	2.61^{a}	22.72^{cd}	0.76^{bc}	3.65^{cd}	11.38^{d}	1.14^{b}	23.91^{e}	3.44^{a}	9.77^{c}	6.09^{a}	8.18^{a}	2.23^{bc}	1.40^{c}
S. mellifera	Late DS	2.11^{b}	0.52^{c}	1.02^{c}	23.55^{cd}	1.03^{b}	4.91^{bc}	12.93^{cd}	1.27^{b}	29.75^{b}	1.57^{d}	9.96^{c}	1.95^{e}	3.19^{cde}	3.88^{a}	4.43^{a}
	Early RS	2.54^{ab}	0.90^{b}	1.31^{c}	22.09^{cd}	0.94^{b}	7.62^{a}	11.73^{d}	0.82^{d}	23.48^{e}	2.02^{c}	15.46^{a}	2.28^{de}	3.62^{cde}	3.57^{a}	3.94^{a}
T. sericea	Late DS	1.64^{c}	0.52^{c}	2.09^{b}	26.42^{b}	0.48^{c}	4.29^{c}	16.66^{b}	1.79^{a}	29.60^{b}	2.11^{c}	6.75^{d}	2.40^{de}	2.48^{de}	1.61^{c}	2.70^{b}
	Early RS	1.17^{c}	1.17^{ab}	1.89^{b}	28.36^{a}	0.73^{b}	4.15^{c}	19.84^{a}	1.97^{a}	24.37^{e}	2.24^{c}	5.03^{d}	2.48^{d}	2.12^{e}	2.00^{bc}	3.59^{ab}
SEM		0.13	0.08	0.11	0.51	0.13	0.28	0.35	0.08	0.24	0.05	0.59	0.14	0.31	0.23	0.25
P value	Species	0.0052	0.0454	0.0002	0.0014	0.0695	0.002	0.0001	0.0006	<0.0001	<0.0001	0.0013	<0.0001	0.0005	0.0025	0.0007
	Season	0.2781	0.0005	0.4846	0.1740	0.8207	0.0025	0.0057	0.2080	<0.0001	<0.0001	0.0521	0.0013	0.0071	0.0388	0.2313
	Species x Season	0.0693	0.1194	0.0501	0.1075	0.5593	0.0222	0.0015	0.0565	0.0012	0.0024	0.0089	0.0180	0.0303	0.0779	0.1190
R-square		0.98	0.97	0.99	0.98	0.84	0.98	0.99	0.98	1.00	1.00	0.98	1.00	0.99	0.97	0.98

TFA = total fatty acids; C12:0 (lauric acid); C14:0 (myristic acid); C16:0 (palmitic acid); C17:0 (heptadecanoic acid); C18:0 (stearic acid); C20:0 (arachidic acid); C22:0 (behenic acid); C24:0 (lignoceric acid); C26:0 (cerotic acid); C28:0 (montanic acid); C18:1n9 (oleic acid);C18:1c11 (*cis*-vaccenic acid); C18:2n6 (linoleic acid); C18:3n3 (α-linolenic acid); SEM = Standard error of mean; $^{a-e}$Means with different superscripts within a column differ significantly **(**P < 0.05**)**

3.5 Discussion

3.5.1 Chemical composition

The nutrient factors considered in this study can be grouped into 2 categories as suggested by Naumann *et al*. (2017): (a) nutrients (including protein fractions, ether extract, ash); and (b) fibre (including cellulose, hemicelluloses and lignin), which influence the physical toughness as well as digestibility. According to Mnisi and Mlambo (2017), the nutritional value of bush species varies widely depending on a number of factors, which require prior investigation to ensure efficient utilization.

In this study, the species x season interactions were significant on all chemical composition variables. The effects of species and season have also been highlighted by Theart *et al*. (2015) and Papachristou *et al*. (2005). Theart *et al*. (2015) further emphasized that seasonal variations on chemical compositions are mainly attributed to the physiological changes which occur in plants during the growing season. On the other hand, González-Andrés and Ceresuela (1998), stressed that species also vary in their response to climatic and physiological changes and these differences determine their actual nutrient value as forage.

As far as nutrients are concerned, DM, OM, EE and CP of all species were generally higher in early rainy season then dropped in the late rainy and dry seasons. This observation may partly be explained by the change in ratio of leaves to stems in favour of the rainy season, as recurrent flushes during the year are closely coupled to rainfall (Scogings *et al*., 2004). Hence, nutrient concentrations within species would be expected to fluctuate significantly and in relation to the growth flushes. It was observed that during the late winter season harvest, bushes had shed most leaves and the new ones had not sprouted yet, or just started sprouting. Larbi *et al*. (1997) also shared the same sentiment that nutrients such as CP tend to be higher in leaves and young stems than in other parts of the plant, which in this case could also potentially indicate better nutritional quality of those species in the early rainy season than in the dry season. Similarly, in the late rainy season, leaves had matured and were getting into senescence as winter approached.

It was, however, observed that while *S. mellifera* and *D. cinerea* were already almost in full leaf in December (early rainy season) harvest, *T. sericea* and *R. trichotomum* still had no signs of new-season leaves. In line with this, the rainfall records for 2017/2018 and 2018 seasons presented in Figures 3.2 and 3.3 respectively, indicated that JAP where *S. mellifera* and *D. cinerea* were harvested received good rainfall than other study areas (SAN and KAL) from which *T. sericea* and *R. trichotomum,* were harvested. This could possibly explain the observed absence of new-season leaves on *T. sericea* and *R. trichotomum* during the early rainy seasons (December 2018) harvest, which could have eventually led to species by season interaction on the nutrients concentration observed in this study. Katjiua and Ward (2007) reported flushing of new-season leaves as early as October on *T. sericea* in the same agro ecological zone. They further emphasised that the timing of pre-rain flushing of leaves was determined by the amount of rain received during the preceding rainy season and if good rains were received, flushing of new leaves could even start as early as late September.

The seasonal variation was more prominent on CP for *S. mellifera* followed by *D. cinerea* which were significantly higher in all three seasons than for the other two species. The seasonal pattern in CP was in agreement with the trend observed by Abdulrazak *et al*. (2000), Marius (2016) and Honsbein *et al*. (2017). Honsbein *et al.* (2017) reported that during rainy season, when bushes had abundant leaves, the CP content of *S. mellifera* was 124.0 g/kg DM, compared to 64.7 CP g/kg DM in dry season, which is in agreement with values obtained in this study. Increased lignification as the growing season progresses from the early to late rainy season, could contribute to the reduced CP concentration observed.

Apart from seasonal variation, the differences in CP content among species within a season can be explained by inherent characteristics of each species related to the ability to extract and accumulate nutrients from soil and/or to fix atmospheric nitrogen, in the case of leguminous bush plants (González-Andrés and Ceresuela, 1998; Njidda, 2011). Biological nitrogen fixation particularly during the early rain season increases CP (Holmes *et al*., 2007), which may explain the observed increase CP in *S. mellifera*. The significant higher CP contents of *S. mellifera* in both seasons compared to the other three species could also be due to its inherent physiological characteristics. *Senegalia mellifera* tends to flower earlier before the new-season leaves sprout, than the other three species considered in this study. Hence, the flowers would also be expected to contribute positively to the nutritional value of its remaining dry leaves in winter compared to others. In addition, the type of bush species and its growth cycle development also differs according to a given climatic condition (amount of rain received, soil nutrients and temperature) and geographical area. For example, *R. trichotomum* is a native deciduous dwarf shrub that is only found in drier regions of Namibia (KAL) (Giess, 1998).

Generally, the CP contents of *S. mellifera* observed in this study were lower compared to those reported by Nassoro *et al*. (2014), Abdulrazak *et al*. (2000) and Osuga *et al.* (2008) in leaves; Dambe *et al*. (2015) and Theart *et al.* (2015) in leaves with twigs. The obtained CP values for *D. cinerea* were also lower than those reported by Kasale (2013) and Marius (2016) in leaves; Theart *et al*. (2015) in leaves with twigs of *D. cinerea*; while the CP for *R. trichotomum* compares favourably with the figure reported by Marius (2016) in leaves. In this study, *T. sericea* had the lowest CP contents of 46.9 and 47.9 g/kg DM in late dry and early rainy season respectively, which were much below the 100.3 g/kg DM reported by Marius (2016) and 97.4 g/kg DM by Theart *et al*. (2015). This could be attributed to the dilution effects of a large proportion of wood (branches) included in the forage samples in this study as compared to only leaves or leaves with small twigs used in those other reported studies.

With regards to fibre, irrespective of the season, all fibre fractions i.e. NDF, ADF and ADL in this study (Table 1) were higher for all species than for those of the same species reported by Kasale (2013), Theart *et al*. (2015) and Marius (2016) who mainly sampled leaves or leaves with small twigs of 15 mm stem diameter. High fibre fractions and lignin, are generally more common in most tropical bush shrubs than in herbaceous plants (Njidda, 2011), but the content varies according to species, stage of growth, age and the plant parts.

Exceptionally high fibre fractions observed in this study was expected because of the higher stem: leaf proportion included in bush samples. These results were in agreement with the observations by Larbi *et al*. (1997), who emphasised that leaves contain less cell wall fractions compared to stems. The stem size harvested or included in bush samples of this study was done to mimic the current practice of producing milled bush feed in Namibia. The process involves harvesting and milling bush leaves and branches with a diameter of at most 20 mm ("broom stick size") as reported by Honsbein *et al*. (2017).

Likewise, fibre fractions varied markedly between seasons and among plant species. The inconsistency in the seasonal variation within species, could be associated with their phenological variation in their response to climatic changes in different seasons (Larbi *et al*., 1997; González-Andrés and Cineresuela, 1998). This observation could be interpreted as the absence or presence of new flushes which possibly dilute the high fibre fractions in the stem (Scogings *et al*., 2004). In this case, for example, *R. trichotomum* and *T. sericea* further lost even the little dry leaves left in the later dry season and were mainly stems without any leaves in December (early rainy season), due to drought. Hence, the observed increased NDF and ADL in relation to the other two species. Also consistent with expectations, Scogings *et al*. (2004) observed lower cellulose and lignin in other bush species (*Grewia occidentalis*, *Scutia myrtina*, *Diospyros lycioides*, *Rhus longispina*, *Ehretia rigida* and *Acacia karroo*) during growth than during plants dormancy. *Rhigozum trichotomum* and *T. sericea* sprouted late compared to other species due to delayed rains in their respective study areas, which possibly explain the increase in their concentration of some nutrients (CP and EE) in the late rain season, as they are mostly concentrated in the leaves.

3.5.2 ***Protein fractions***

The protein fractions of all species considered in this study varied widely among species and by season. This was in line with the findings by Fall-Toure and Michalet-Doreau, (1995), who also observed great variations in the protein fractions of different browse species. Soluble protein (faction A + B1) which is generally assumed to be rapidly degradable in the rumen (Licitra *et al*., 1996), comprises mostly of NPN compounds like ammonia, urea, nitrates, amino acids as well as some small peptides and true protein. Of the species considered in this study, *S. mellifera* had the most soluble protein followed by

R. trichotomum, then *T. sericea,* while *D. cinerea* had the least fraction of soluble protein in both seasons.

The observed seasonal variation in protein fractions may partly be explained by the increase in ratio of leaves to stems during the rainy season, as new flushes during the year are closely linked to rainfall (Scogings *et al*., 2004). In addition to seasonal variation, the differences observed in nitrogen content among species within a season could also be explained by inherent characteristics of each species related to the ability to extract and accumulate nutrients from soil and/or to fix atmospheric nitrogen, in the case of leguminous bush plants (González-Andrés and Ceresuela, 1998; Njidda, 2011). For example, the significant higher nitrogen contents of *S. mellifera* in both seasons compared to the other three species could be due to its inherent physiological characteristics of being leguminous and also it tends to flower earlier even before the new-season leaves sprout. In that case, the flowers would also be expected to contribute positively to the nutritional value of its remaining dry leaves in winter. Traxler *et al*. (1998) also highlighted other species related attributes such as possible changing in the fibre content and the extent to which it binds nitrogen. In this study, NPN represented a higher proportion of soluble protein for all the species in both season, in relation to STP. According to Amburgh *et al*. (2010) and Brandstetter *et al*. (2019), NPN is fundamental for good ruminal functioning, because the rumen microbes responsible for fermenting structural carbohydrate use NPN, mostly ammonia as a source of nitrogen for microbial protein synthesis.

The protein fractions NDSP (intermediate degraded fraction) and ADSP (very slow degraded fraction) are associated with the cell wall of the plant and are slowly available, leaving the unavailable fraction ADIP (O'Connor *et al*., 1993). The study further demonstrated that it appears that when the passage rate of the fibrous fraction is very fast or the degradation rate is very slow, then this fraction would escape degradation in the rumen. This implies that unlike NPN, which is assumed to be completely degraded, the rates of degradation of B protein fractions are highly variable and can result in variable amounts of protein being degraded in the rumen. This may have further implications on the utilization of dietary CP by the animal, and may not be as efficient as expected. The values of NDSP-ADSP for bush species in this study ranged from 4.99 to 27.69 and 0.66

to 20.95 g/100g CP respectively, which may be an indication of their limited contribution to the overall available CP, similar to those observed in literature (Fall-Toure and Michalet-Doreau,1995; Shayo and Udén, 1999) in browse species.

With regards to the C fraction of protein bound to fibre as ADIP, values ranged from 23.73 to 52.47 g/100g CP, which was found to be highest in *T. sericea*. These values were higher than those observed by Fall-Toure and Michalet-Doreau (1995), in the range of 5 to 35 g/100g CP. The ADIP which is generally considered indigestible may include proteins and nitrogen compounds associated with lignin (Fall-Toure and Michalet-Doreau, 1995; Brandstetter *et al*., 2019) and tannin-protein complexes (Van Soest *et al*., 1991; Fall-Toure and Michalet-Doreau, 1995; Brandstetter *et al*., 2019) which makes them highly resistant to the degradation by microbial enzymes. Mahesh *et al*. (2017) in their study demonstrated that higher acid detergent insoluble protein (ADIP) content decreased intestinal nitrogen digestion and the total available N. The quantities of tannins and phenolic compounds in browse species vary widely (Fall-Toure and Michalet-Doreau, 1995), which could be the cause of variation in the ADIN among the browse species. The other possibility for the high fibre bound protein observed in this study could also be related to the inclusion of thick branches in harvested samples, as opposed to the lower values observed by Abdulrazak *et al*. (2000) and Osuga *et al*. (2008) in leaves of some *Vachellia* (formerly *Acacia)* species.

3.5.3 ***Anti-nutritional factors: Total Phenols and tannins***

With the exception of *D. cinerea*, the values of TP and TT for other species were in line with those reported by Osuga *et al*. (2008) in a range of 15.1 to 72.2 g/kg DM and 2.4–34.8 g/kg DM, respectively, of some browse species in Kenya. In another study in Ethiopia, the concentration of TP of some browse species was in the range of 3.7 to 70.6 g/kg DM, while TT was in the range of 2.5 to 68.1 g/kg DM (Mengistu *et al*., 2017), which is in line with this study. However, Rubanza *et al*. (2005) observed much higher levels of TP and TT than in this study, which ranged from 99 to 281 g/kg DM and 84 to 256 g/kg DM of six *Acacia* species from Tanzania.

The concentrations of TP and TT for *T. sericea* in the current study were exceptionally much higher than those of the other bush species. However, Njidda (2011) reported that total phenolics in plants can reach up to 40% of the dry matter and the levels for the species in this study fall below this figure. It appears from this study that TT constituted a large portion > 60% of the TP, which is the opposite in grasses, where the major phenolic is lignin that is bound to all plant cell walls, and is a significant limiting factor in their digestion in the rumen (Njidda, 2011).

Total tannins are further fractioned into CT and HT which have been reported to differ in their nutritional and toxic effects to the animals. According to Akande *et al.* (2010) the CT have more profound digestibility-reducing effect than HT, whereas, the latter may cause varied toxic manifestations due to hydrolysis in rumen and from where they get absorbed in the bloodstream. In this study, the concentration of CT for all the species ranged from 1.86 to 24.82 g/kg DM across the seasons, while the HT ranged from 1.55 to 170.84 g/kg DM. With the exception of HT in *T. sericea* during the early rainy season, all species had lower CT and HT than 40 g/kg DM reported to be the upper beneficial level in animal feeding and nutrition (Rubanza *et al*., 2005; Osuga *et al*., 2008), hence negligible negative effects could be expected from feeding milled bush feed of these species. The greatest tannins concentrations for *T. sericea* during the rainy season were also reported by Cooper *et al*. (2014) and Naumann *et al*. (2017) compared to other Southern African Savannah browse species evaluated in those studies.

Indeed, Salem (2005) and Akande *et al.* (2010) indicated that at lower concentration levels of 2-3% CT in ruminant diets, tannins have been reported to be more of beneficial than deleterious and have two general qualities that are relevant to grazing ruminants. They reduce the degradation of useful protein in the rumen by forming protein- tannin complexes to supply the needed amino acids from small intestines (McMahon *et al.*, 2000; Makkar, 2010). The tannin-protein complexes are dissociated under the acidic conditions of the abomasum (pH 2.5 to 3.5) and in alkaline conditions of the intestines (pH < 7.5), releasing protein, availing it for digestion and absorbtion (Mueller-Harvey, 2006). In addition, they also prevent bloat (McMahon *et al.*, 2000) and suppress internal parasites (Hoste *et al*., 2006). However, at higher concentrations (more than 6% DM), CT were reported to reduce intake, digestibility and absorption of nutrients from the gastro intestinal tract (Njidda, 2011). Tannins can have adverse or beneficial effects on herbivores depending on their type and concentration (Min *et al*., 2003; Piluzza and Billutta, 2010) in forages. The CT concentrations of the species in this study were below the critical threshold of 55 g/kg DM that reduces feed intake and live weight gain (Waghorn and McNabb, 2003), although other aspects (type of tannin, chemical structure of CT and diet selection) need to be considered.

The seasonal variations in the phenolics and tannins concentrations observed in this study could be due to climatic and physiological changes in browse plants which consequently induce changes in chemical composition and, in particular, in concentrations of those secondary compounds (Rubanza *et al*., 2005). The higher temperature during the early and late rainy seasons may have exposed plants to stress conditions that could increase biosynthesis of polyphenolic compounds (Fallovo *et al*., 2011). It is also possible that increased moisture during the growing season may have favoured multiplication of arbuscular mycorrhizal fungi (AMF), and tannins are increased in presence of AMF, where they are involved in different mechanisms for resistance against microorganisms (Cheynier *et al*., 2013).

Differences in levels of phenolics and tannins among species in this study could be explained by differences in genotypic factors that control biosynthesis and accumulation of polyphenolic compounds in browse plants (Rubanza *et al*., 2005; Mengistu *et al*., 2017). Slight variations between observed and literature values in phenolic and tannin contents could be attributed to the nature of tannin in different browse species, the influence of soil and climatic factors on accumulation of polyphenolic compounds in plants, stage of growth and the proportion of leaf sample harvested (Abdulrazak *et al*., 2000; Rubanza *et al*., 2005; Aliyu *et al*., 2016).

3.5.4 **Macro and micro minerals**

The observed variation in the content of individual minerals among bush species in this study could be as a result of inherent characteristics of each species related to its ability to absorb and or accumulate minerals coupled with soil factors such as type and fertility, similar to what was reported by Abdulrazak *et al*. (2000). A distinct seasonal fluctuation in certain mineral concentrations was also observed in some species, generally with an increased peak in early rainy season and a decrease in late rainy and or late dry seasons. This may reflect seasonal changes in physiological reactions in plants due to climatic conditions and suggest that mineral concentration decrease as plants loose their leaves and dry off (Aganga and Mesho, 2008).

The levels of most minerals reported for species in this study,were lower than those of other browse, specifically *Acacia* species reported by Abdulrazak *et al.* (2000); *V. karroo* reported by Mapiye *et al*. (2011); indigenous browse from Botswana including *T. sericea* and *D. cinerea* by Aganga and Mesho (2008), indigenous browse from Botswana including *S. mellifera* (Dambe *et al*., 2015); indigenous browse in Nigeria (Ogunbosoye *et al*., 2015) indigenous browse in Ethiopia (Derero and Kitaw, 2018). However, Ca and P content of species in this study were within the values of the same species reported by Marius (2016). Though the Fe contents were exceptionally high in the late dry season, especially for *T. sericea*, they were comparable with those reported for some species by Derero and Kitaw (2018). The differences in the concentrations of minerals in the current study from those reported by others could be due to species differences, growth stage and or season of sampling, soil conditions, plant part (leaves, twigs) and leaf to stem fractions harvested.

The Ca concentrations in the species were within the levels recommended for beef cattle (2 g/kg DM) and sheep (2.1 - 4.2 g/kg DM), but P contents were far below the minimum requirements for beef cattle and sheep at 3.1 - 4.0 g/kg DM and 1.6 - 3.2 g/kg DM respectively as reported by Nassoro (2014). Based on recommendations of 2.0 g/kg DM Mg and 70 g/kg DM K in the diet of ruminants reported by Ogunbosoye *et al*. (2015), all bush species considered in this study had insufficient levels of Mg and K.

Regarding micro minerals, levels were sufficient to meet the minimum ruminants requirements of 30 - 60 mg/kg DM for Fe; beef cattle (20 - 50 mg/kg DM), sheep (20 - 40 mg/kg DM) and goats (> 5 mg/kg DM) for Mn as reported by Ogunbosoye *et al*. (2015). However, the Zn levels were below minimum requirements for ruminant diets of 20 - 40 mg/kg DM, while Cu levels could meet requirements of sheep and goats diets (0.1 mg/kg DM) but not for beef cattle (4 - 10 mg/kg DM) (Nassoro, 2014).

Another critical aspect related to minerals, is the ratio of calcium to phosphorus. It is generally known that Ca is closely related to P metabolism in the formation of bones and a Ca:P ratio of 2:1 is recommended (Abdulrazak *et al*., 2000). Due to low levels of P observed in this study, the Ca:P ratio ranged from 9:1 to 20:1, which is slightly higher than ratios reported by Abdulrazak *et al*. (2000), but lower that the one reported by Topps (1992). Feed formulation should hence ensure a more favourable Ca:P ratio to meet the requirements of ruminants.

3.5.5 **Amino acids**

Higher concentrations of total and individual amino acids observed in *S. mellifera* and *D. cinerea* compared to the other two species seem to be also reflected in their observed higher percentage of crude protein. The difference in amino acids composition among species can be explained by inherent characteristics of each species in relations to its ability to extract and accumulate nutrients from soil and/or to fix atmospheric nitrogen, which is the case for leguminous plants (González-Andrés and Ceresuela, 1998; Njidda, 2011). The other possible factor causing variation in the amino acid composition of species under study may be soil type as related to the different geographical locations where species were harvested.

The seasonal variation could be attributed to the moisture availability which facilitates absorption and/or synthesis of nutrients. For example, the significant increase in the amino acids composition of *S. mellifera* and *D. cinerea* in the early rainy season could be associated with the amount of rainfall received in the areas where they were harvested as compared to other sites (Figure 3.2 and Figure 3.3). Hence, species x season interaction effects impact on both the quality and quantity of CP in addition to altering the amino acid profiles.

The contents of amino acids of species reported in this study were lower than those of leaf meals from some indigenous browse species (*V. nilotica*, *V. karroo* and *C. mopane*) reported by Hlatini *et al*. (2018). These differences in the amino acids contents could be explained by a number of factors including species, soil type (location), plant part (leaf, twigs and stem), age of leaf and season of harvest.

3.5.6 **Fatty acids**

The finding of this study conformed to the observations by Boufaïed *et al*. (2003), Bouazza *et al*. (2012), Goossen *et al*. (2018) and Acar *et al*. (2019) that FA composition of forages is typically dominated by high proportions of five fatty acids: α-linolenic acid, linoleic acid, oleic acid, stearic acid and palmitic acid. The main factors reported to be influencing the FA content and composition of forages are species, vegetation stage, season and conditions of conservation (Boufaïed *et al*., 2003; Bouazza *et al*., 2012; Goossen *et al*., 2018).

Fatty acid composition varied markedly among seasons and bush species. While all bush species in this study contained significant percentages of C18:2*n*-6, their concentrations dropped drastically during the early rainy season in which plants were expected to be in the vegetative stage, with increasing proportion of leaves to stem than in the late dry season. This observation was in contrast to suggestions by Boufaïed *et al*. (2003) and Dewhurst *et al*. (2001) that FA in forages are predominantly of leaf origin and possible higher proportion of leaves to stem could have led to higher FA concentration.

The inconsistencies in the seasonal variation within and among species were also observed for C18:2, C16:0, C18:3*n*-3 and C18:0, which could be associated with the species phenological variation in their response to climatic changes in different seasons. For example, the observed high concentration of C18:3*n*-3 and C18:0 for *D. cinerea* and *S. mellifera* in contrast to *T. sericea* and *R. trichotomum* in the early rainy season could possibly be as a result of increased leaf-stem ratio, as observed by Boufaïed *et al*. (2003) in other forages. Leaves are higher in membrane lipids compared to stems and thus would have higher concentrations of C18;3 during the early vegetative stage (Dewhurst *et al*., 2001). The higher C18:3*n*-3 and C18:0 in *D.cinerea* and *S. mellifera* in contrast *T. sericea* and *R. trichotomum* could also be interpreted as the absence or presence of new flushes which possibly dilute the high fibre fractions in the stem (Scogings *et al*., 2004). This was in agreement with physical observations made during the December (early rainy season) harvest that while *S. mellifera* and *D. cinerea* were already almost in full leaf, *T. sericea* and *R. trichotomum* still had no signs of new-season leaves. In line with that, Scogings *et al*. (2004) highlighted that recurrent flushes during the year are closely coupled to rainfall. Hence, the rainfall records presented in Figures 3.2 and 3.3, both in 2017/2018 and 2018 rainfall seasons, in respective study areas could possibly point to those variations.

However, the season x species interactions was observed in the case of C18:1c9, C16:0 which only increased for *T. sericea* in the early rainy season, even with absence of new leaves. This could possibly be related to the genetic features of a specific bush species and its growth cycle development, in a given climatic condition and geographical area, which may not necessarily only depend on the amount of rain received and or change in temperature but may also be due to soil fertility. Similarly, the later explanation could also be a possible reason for the observed four FAs: myristoleic (C14:1c9), palmitoleic (C16:1c9), pentadecylic (C15:0) and heneicosanoic (C21:0) acids, which were only found in *R. trichotomum.* In this case, the fact that *R. trichotomum* is a native deciduous dwarf shrub dominantly found in drier regions of Namibia (KAL) (Giess, 1998), could give it unique inherent physiological features or responses that are not found in other plants.

It should however, be noted that the concentrations of all dominant FA for bushes in this study were lower than those of leaf meals from indigenous browse such as *A. Karroo* reported by Mapiye *et al*., (2011), and some grasses and legumes (Boufaïed *et al*., 2003; Bouazza *et al*., 2012). This could be attributed to the species differences and dilution effects by a large proportion of wood (branches) included in the forage samples of this study as compared to only leaves or young whole plants from their studies. However, the concentrations of C16:0, C18:0, C18:1 and C18:2 for all species in this study were higher than those of Caramba hay (*Lolium multiflorum cv. Caramba*) reported by Acar *et al*. (2019), except for concentration C18:3, which was lower than theirs. This may imply that, in the absence of other quality fodder, milled bush would still serve as source of major unsaturated fatty acids. The presence of FAs in the four encroacher bush species could modify rumen fermentation (Newbold *et al*., 2004). The C18:3 is the main FA in forages and is the most important in enhancing meat and milk quality (Glasser *et al.,* 2013), hence harvesting bush biomass in the early vegetative stage would enhance animal product quality.

3.6 Conclusion

The species by season interaction for chemical composition underscores the importance of diet diversity in meeting the nutritional requirements through formulated feed. We reject the hypothesis of similarity in chemical composition, ANFs, amino acid profile, minerals and FAs across the species and seasons; these components will depend on the species x season interactions. This implies that timing of harvesting is crucial in ensuring optimal chemical composition of bush-based feed. A balanced proportion of soluble protein (Fraction A + B1) presented by *R. trichotomum* and *S. mellifera* could promote good ruminal functioning compared to other species which had a large portion of their CP bound to fibre. The levels of anti-nutritional factors obtained were relatively low and within safe limits, thus making these bushes safe and suitable for animal feeding, except for *T. sericea* which presented high levels of HT during the early and late rainy season, respectively. The concentration of most macro and micro minerals are also sufficient for livestock maintenance except for P, Na, Cu and Zn. All species had relatively low total amino acids and fatty acids but presented an appreciable profile of essential fatty acids which are associated with good fatty acids in animal products. The overall nutritional profile of these species indicates that they could be potential fodder resources for ruminants.

CHAPTER 4

In situ neutral detergent fibre digestibility, *in vitro* organic matter digestibility and methane production of four Namibian encroacher bush species

4.1 Abstract

Encroacher bush species have been undervalued due to insufficient information about their potential feeding value. This study evaluated the *in situ* neutral detergent fibre (NDF) digestibility, *in vitro* organic matter digestibility (OMD) and methane production of *Senegalia mellifera, Dichrostachys cinerea, Terminalia sericea* and *Rhigozum trichotomum*. Leaves and twigs (< 20mm) were harvested during late dry and early rainy seasons. Except for *S. mellifera*, the *in vitro* OMD and digestible organic matter in dry matter (D-value) of other species decreased ($P < 0.001$) from late dry to early rainy season. *Dichrostachys cinerea* recorded the lowest ($P < 0.001$) *in vitro* OMD and D-value in both seasons compared to other species. Increasing indigestible NDF (iNDF) contents ($P < 0.001$) were observed from late dry to early rainy season for all species, except for *S. mellifera* which decreased. The iNDF values ranged from 734 to 915 g/kg NDF with *D. cinerea* having the highest and *T. sericea* having the lowest content in both seasons. *In vitro* methane gas production of all four species was higher ($P = 0.0004$) during the late dry season compared to the early rainy season (147.6 versus 92.0 mL/g DM). Seasonal variability in fibre and phenolics may affect digestibility and methane production of the four encroacher bush species. In conclusion, based on the low OMD and high iNDF, the studied species may require further intervention to improve their digestibility and feeding value.

4.2 Introduction

Browse plants, in general, constitute an important feed resource for ruminants because they provide considerable amount of biomass from leaves and small twigs of less than 20 mm as well as flowers and pods. In addition, they maintain their nutrients into the dry season when other feed resources such as grasses are depleted and are of low nutritional quality (Moleele, 1998; Salem, 2005; Quansah and Makkar, 2012). However, rangeland habitats become inaccessible to livestock as browse due to the encroaching nature of some species. This results in the reduction of the rangeland carrying capacity and productivity by up to two thirds. The situation further causes economic loss every year to the livestock industry and agricultural sector in general, estimated to be more than one billion Namibian dollars (MAWF, 2012).

In recent years, research attention has been diverted from the removal of encroacher bushes as weeds, to their utilisation as animal feed (Mapiye *et al*., 2011). Widespread adoption of feeding ruminants on bush-based rations from encroacher bushes has been gaining momentum in Namibia (Honsbein *et al*., 2018), both as a drought relief strategy and also for commercial purposes. There is still, however, a need for an adequate understanding of the feeding potential of encroacher species when harvested as milled bush, in order to ensure their approximate use in ruminant nutrition.

Based on their intensive research with *Senegalia mellifera*, Honsbein *et al*. (2018) concluded that milled bush was generally characterized by its high dry matter (DM) yield, but required supplementation with additional concentrates to reduce productivity losses due to low feed intake. This implies that milled bush is mainly composed of the structural carbohydrates neutral detergent fibre (NDF), which largely influences its digestibility. However, other researchers (Daniel *et al*., 2014; Harper and McNeill, 2015) have reported that DM and fibre digestibility is directly related to the potentially digestible portion of NDF (pdNDF). They defined the latter as the NDF fraction that disappeared after a long incubation period, leaving the indigestible component of NDF (iNDF) which is unavailable for microbial digestion. Hence, understanding the dynamics of fibre digestibility is key to minimizing the inhibitory effects of NDF on feed intake of bush-based feed. Furthermore, Harper and McNeill (2015) reported that iNDF is a useful index for predicting OM digestibility and its inclusion in feed evaluation models increases accuracy.

Other factors associated with the feeding value of forages is the production of greenhouse gases (GHG) particularly methane. The production of methane by livestock and its impact on the environment is a worldwide concern because it contributes to global warming (Moss *et al*., 2000; Goel and Makkar, 2012; Broucek, 2014; Brouček, 2015; Theart *et al*., 2015; Gemeda and Hassen, 2018; Aboagye and Beauchemin, 2019). Methane (CH_4) production is increased when feeds high in fibre are fed to ruminants (Goel and Makkar, 2012). Considering that milled bush is also high in fibre, there is limited information on the methane production of these bush species. The objective of the study was therefore to evaluate the *in situ* NDF digestibility, *in vitro* OM digestibility and methane production of *Senegalia mellifera*, *Dichrostachys cinerea*, *Terminalia sericea* and *Rhigozum trichotomum*.

4.3 Materials and Methods

The detailed procedures on sampling and preparations of materials are outlined in Sections 3.2.1 to 3.3.3 of Chapter 3.

4.3.1 *In situ* neutral detergent fibre digestibility

The indigestible neutral detergent fibre (iNDF) and digestible neutral detergent fibre (dNDF) were determined by the *in sacco* nylon bag technique, after incubating nylon bags with bush samples in the rumen of two non-lactating Finnish Ayrshire cows fitted with rumen cannula for a period of 12 days (Ahvenjärvi *et al*., 2000; Nousiainen et al., 2003). The two cows used in the experiment were fed with grass hay plus minerals. The nylon bags (Sefar Petex®, Switzerland; external dimensions 60 × 120 mm) with small pore size of 17 µm were used to minimize particle inflow and outflow, but still ensuring sufficient microbial activities inside the bags (Jančík *et al*., 2008).

A sample weight of 4 g was weighed into each nylon bag and incubated in duplicates in each cow, as shown in Figure 10.4 in Appendix. The bags were collected from the rumen after the incubation period of 12 days, first rinsed by hand and then in the washing machine with cold water for 1 hour. Following the procedure described by Ahvenjärvi *et al*. (2000)

and Nousiainen *et al*. (2003), the same bags with samples were dried at 100ºC in a forced oven for 48 hours. These samples were further weighed and boiled in neutral detergent solution for 1 hour using a cooking pot to remove microbial and endogenous matter. The samples were again rinsed in cold water by hand and then dried at 100ºC for 48 hours and weighed. The iNDF concentration (g/kg DM) were expressed as NDF residue after rumen incubation. Digestible NDF (dNDF) (g/kg DM) was calculated as NDF-iNDF.

4.3.2 *In vitro* organic matter digestibility

The *in vitro* organic matter digestibility (OMD) and the content of digestible organic matter in dry matter (D-value) of bush samples were based on the two stage pepsin + cellulase solubility technique as described by Friedel (1990) as modified by Nousiainen *et al*. (2003). Approximately 200 mg of bush samples were ground to pass through a 1 mm sieve and weighed into glass beakers. For the first step, samples were pre-treated with 20 ml pepsin (Merck KGaA, Germany) in 0.1 N HCl solutions and then shaken before incubating at 40°C for 24 hours, using a laboratory conventional oven. After incubation the same sample and solution were heated at 100°C for 10 minutes. The samples were washed with cold water and filtered through sintered grass crucible, porosity No.3. For the second step, 30 ml of cellulase (Cellulase "ONOZUKA" R-10; Yakult Pharmaceutical IND.CO, LTD) in an acetate-acetic acid buffer solution was added to the sintered grass crucibles and placed in beakers and incubated at 40 °C for 48 hours (Fig. 10.2 Appendix). At the end of incubation, the samples were washed with hot distilled water, rinsed with acetone, dried at 100°C overnight (dry weight) and then ashed at 500°C for 3 hours to obtain the indigestible organic matter.

In vitro digestibility values were calculated using the formulas of Huhtanen *et al*. (2006) given below:

OMD = (OMi - OMf)/OMi

D-value = (OMi - OMf)/DMi

Where, OMi: initial amount (g) of organic matter incubated (obtained by subtracting the ash content from the total dried initial sample weight (100%)); OMf: final amount (indigestible residue) of organic matter after incubation process (g); DMi: initial amount

(g) of dry matter incubated (obtained by subtracting the moisture content of the sample from the total initial sample weight (100%).

4.3.3 *In vitro* methane determination

Methane (CH_4)gas production was determined by *in vitro* method using the Gas Endeavour Automatic Gas Flow Measuring System (Bioprocess Control, Lund, Sweden, Fig. 10.3 Appendix), after incubating bush samples with rumen liquor collected from two Finnish Ayrshire dry cows fitted with rumen cannulas. The cows were fed grass silage only plus minerals as the usual maintenance ration for dry cows under that production system. Rumen digesta were collected at 4 different sites in the rumen into plastic bottles and the sealed bottles were placed in warm water bath at 40°C. Rumen digesta was filtered through a 250 µm sieve under nitrogen flush in the laboratory. The buffer (artificial saliva) was prepared following the method of McDougall (1948). The prepared buffer was mixed with rumen liquor in a ratio of 1:2 to make 400 ml of incubation media per incubation bottle.

About 5 g of dried sample were milled through a 1 mm sieve and added to each incubation bottle of 500 ml in size, then incubated at 39°C for 24 hours in triplicates , plus one blank (without feed) to correct for CH_4 production of residual OM in the innoculum. The Gas Endeavour Automatic Gas Flow Measuring System consisted of a sample incubation unit, a gas absorption unit and a gas measuring unit connected to web-based software running on embedded server. An integrated embedded data acquisition system was used to record, display and analyse the results. Methane gas production results were calculated at 0°C, 1 standard atmosphere (atm) and dry gas.

In situ neutral detergent fibre digestibility, *in vitro* organic matter digestibility and *in vitro* methane gas production analyses were carried out at the University of Helsinki, Department of Agricultural Sciences, Finland during the short term research visit, from September 2018 – February 2019 (6 month).

4.3.4 Statistical Analysis

The experiment design was a factorial arrangement with 4 species x 2 seasons for *in situ* neutral detergent fibre digestibility, *in vitro* organic matter and methane gas production.

The third season (May 2019, late rainy season) data for those parameters were not available because the equipment and consumables needed for those analysis were not available in Namibia. Hence, the first two seasons' analysis were done during the six months' short term research visit in Finland. The data for *in vitro* organic matter and *in situ* neutral detergent fibre digestibility were analysed based on 4 x 2 factorial treatment design, and subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure (SAS Institute Inc, 2009). Data on digestible neutral detergent fibre (dNDF) was transformed using logarithms to ensure it had an approximate normal distribution before analysis. The main effects considered in the model were bush species, season and their interaction. The following statistical model was used:

$Y_{ijk} = \mu + F_i + S_j + FS_{ij} + e_{ijk}$

Where:

Y_{ijk} = dependent variable (*in vitro* OMD, iNDF, dNDF, etc.);

μ = overall mean;

F_i = effect of browse species (i= 1, 2, 3, 4);

S_j = effect of season (j = 1, 2);

$(FS)_{ij}$ = interaction effect of browse species and season;

e_{ijk} = random component explaining variation among experimental units (EU) on the same treatment.

For the statistical analysis, seasons were defined as late dry season [LDS] (August – November); early rainy season [ERS] (December – March). Means were separated using the Tukeys' Studentised range test. Differences among means with $P < 0.05$ were considered significant. In addition, differences among means with $0.05 \leq P \leq 0.10$ were regarded as representing tendencies.

4.4 Results

4.4.1 *In situ* neutral detergent fibre digestibility

The *in situ* NDF digestibility parameters of the four encroacher species are presented in Table 4.1. There was a significant ($P < 0.0001$) species x season interaction on the *i*NDF. The iNDF_DM for *D. cinerea* and *S. mellifera* decreased ($P < 0.0001$) as season progressed from late dry to early rainy season, while that of *R. trichotomum* and *T. sericea* increased. *Terminalia sericea* and *S. mellifera* had lower iNDF_DM and iNDF_NDF compared to the other two species.

The digestible NDF (dNDF) for all species decreased (P < 0.0001) from dry season to early rainy season, except for *S. mellifera*. Lower (P < 0.0010) digestible NDF (dNDF) values were observed for *D. cinerea* and *R. trichotomum* compared to the other species

4.4.2 ***In vitro* organic matter digestibility**

Species and season interacted (P < 0.0001) in affecting the *in vitro* OMD and D-value (Table 4.1) of the four browse species. Except for *S. mellifera,* the *in vitro* OMD and D-value for other species decreased (P < 0.0001) from dry season to early rainy season. Lower (P < 0.001) *in vitro* OMD and D-value were observed for *D. cinerea* in both seasons compared to other species. The D-value also followed the same pattern as the *in vitro* OMD in both seasons.

Table 4.1 *In situ* NDF and *in vitro* organic matter digestibility of four encroacher bush species collected in two different seasons

		components					
Bush species	**Season**	**dNDF_DM** (g/kg DM)	**dNDF_NDF** (g/kg NDF)	**iNDF_DM** (g/kg DM)	**iNDF_NDF** (g/kg NDF)	*in vitro* **OMD** (g/kg OM)	**D value** (g/kg DM)
D. cinerea	Late DS	99.5^{d}	139.6^{d}	631.0^{a}	859.1^{c}	313.5^{d}	303.7^{e}
	Early RS	81.5^{e}	118.6^{e}	605.5^{b}	879.1^{b}	294.7^{e}	283.5^{f}
R. trichotomum	Late DS	73.7^{f}	126.2^{e}	518.9^{d}	872.8^{b}	424.0^{a}	407.5^{a}
	Early RS	49.9^{g}	83.4^{f}	587.2^{c}	915.2^{a}	383.8^{c}	370.5^{c}
S. mellifera	Late DS	170.7^{a}	255.4^{b}	496.4^{e}	743.5^{e}	411.0^{b}	388.1^{b}
	Early RS	169.0^{a}	261.1^{ab}	477.8^{f}	737.9^{ef}	424.4^{a}	402.8^{a}
T. sericea	Late DS	$163..2^{b}$	264.3^{a}	453.7^{h}	735.0^{f}	405.5^{b}	378.0^{c}
	Early RS	151.9^{c}	245.4^{c}	466.5^{g}	754.1^{d}	384.1^{c}	360.2^{d}
SEM		1.806	2.572	2.742	2.572	3.115	2.809
P-value	Species	<0.0001	<0.0001	<0.0001	<0.0001	0.0157	0.0190
	Season	<0.0001	<0.0001	<0.0001	<0.0001	0.2285	0.2583
	Species x Season	<0.0001	<0.0001	<0.0001	<0.001	<0.0001	<0.0001
R-square		0.95	0.96	0.94	0.96	0.94	0.94

DM = dry matter; NDF = neutral detergent fibre; iNDF_DM = indigestible neutral detergent fibre in DM; iNDF_NDF = indigestible neutral detergent fibre in NDF; dNDF_NDF = digestible neutral detergent fibre in NDF; OMD = organic matter digestibility; OM = organic matter; D-value = digestible OM in DM; SEM = Standard error of means; $^{a-h}$Means with different superscripts within a column differ significantly **(**P < 0.05**)**

4.4.3 *In vitro* Methane gas production

Methane gas production from *in vitro* fermentation was influenced by season (P = 0.0004), presented in Table 4.2. The least square means of methane gas production of digested DM at 24 hours were 132.4 ± 6.6 mL/g for the late dry season and 92.7 ± 6.6 mL/g for the early rainy season.

Table 4.2 Methane gas production at 24 hours (mL/g) of digested DM from four encroacher bush species collected in two different seasons

Bush species	**Season**	**Methane (24h)**
D. cinerea	Late DS	144.9^{a}
	Early RS	91.5^{b}
R. trichotomum	Late DS	108.7^{ab}
	Early RS	92.3^{b}
S. mellifera	Late DS	147.6^{a}
	Early RS	94.8^{b}
T. sericea	Late DS	128.5^{ab}
	Early RS	92.0^{b}
SEM		13.7
P-value	Species	0.4152
	Season	0.0034
	Species xSeason	0.5208
R-square		0.53

$^{a-b}$Means with different superscripts within a column differ significantly (P < 0.05)

SEM = Standard error of means;

4.5 Discussion

4.5.1 *In situ* neutral detergent fibre digestibility

Apart from OM digestibility, the other important concept in forage evaluation is the indigestible NDF (iNDF) of the feed. It appears that a high proportion of the NDF for all species considered in this study was indigestible (≥ 700 g/kg NDF). In addition, there were also seasonal variations, with lower iNDF observed when expressed as a fraction of NDF during dry season than in the early rainy season. Phenolics mostly in leaves and lignin in stems have been widely reported as major factors limiting digestibility of NDF (Woodward and Reed, 1989; Soufizadeh *et al*., 2018). The inclusion of a high proportion of stem parts of 20 mm thickness in the samples of bushes used in this study could be one of the contributing factors to the general high iNDF observed. Harper and McNeill (2015) proposed that the iNDF fraction of forage is attributable to the cross-linking between cell wall lignin and hemicellulose, with the concentration of lignin much higher in stems than in leaves hence limiting the digestion of the NDF fraction in the forage.

It appears that the season by species interaction for iNDF content observed in the bush species could be linked to the nature of the lignin-carbohydrate complex existing in different species (Harper and McNeill, 2015), in combination with their concentration of phenolic contents during different seasons. For example, the high iNDF_NDF observed in the early rainy season compared to the late dry season, could possibly be linked to the increased phenolic contents during the early rainy season (Chapter 3 Table 3.3). Hydrolyzable tannins in particular increased from the late dry season to the early rainy season, but the percentage increase differed by species.

4.5.2 *In vitro* organic matter digestibility

Digestibility is a major determinant of nutritive value of a feed (Dambe *et al*., 2015), and studies on the digestibility of bush fodders are very important as they allow the estimation of nutrients available to the animal (Sanon, 2007). The *in vitro* OM digestibility values of all species in the present study ranged from 294.7 to 424.4 g/kg DM which were considerably lower than those reported by Dambe *et al*. (2015). Dambe *et al*. (2015) found digestibility of woody bush plants in a range of 480 to 660 g/kg DM. The differences among studies could be ascribed to variations in climate as well as different plant species as reported by Jung and Allen (1995) that leaf morphology of plants changes with seasons and within plant species.

Lignin constitutes the main barrier to fibre digestion (Traxler *et al*., 1998) through reduction of enzyme access to cellulose and xylan and adsorption of enzymes, hence reducing rates of hydrolysis of structural polysaccharides (Bansal *et al*., 2009). Lignin adversely affects fibre digestibility because of crosslinks between lignin and cell wall polysaccharides (Jung *et al*., 1997), hence affecting the rate and extent of cell wall degradation (Grabber *et al*., 2004). Cellulose and hemicellulose are the main components of fiber and are intrinsically degradable in the rumen (Adesogan *et al*., 2019). The encroacher bush samples consisted of branches of 20 mm diameter and leaves, but due to the lignification, the degradability of the cellulose and hemicellulose may have been adversely affected, hence limiting utilization of nutrients and energy they contain. Indeed, the ADLom for all species except *S. mellifera* increased ($P < 0.05$) from the late dry season to the early rainy season (Chapter 3 Table 3.1), which corresponds to the decrease in digestibility. In addition to NDF concentration, digestibility of dietary fibre influences dry matter intake (Ruiz *et al*., 1995) and hence productivity. Given the low digestibility of bush feed, it may be desirable to use alkali or enzyme treatment to improve their utilization in ruminant diets (Adesogan *et al*., 2019).

Nutritional composition of rangeland varies by season due to abiotic factors (Mountousis *et al*., 2008). The season by species interaction on OM digestibility observed in this study could be associated with the seasonal variation of protein bound to fibre and condensed tannins in species. Species such as *D. cinerea* that had a combination of large proportion of CP bound to fibre and increasing concentration of condensed tannins in the rainy season, also had low OM digestibility than other species. Camacho *et al*. (2010) reported high digestibility during the rainy season on other tropical bush species. Moreover, in this study, the CP concentration of species as reported in Chapter 3, ranged from 46.91 to 111.79 g/kg DM, but only *D. cinerea* and *S. mellifera* were above 80 g/kg DM. Matlebyane *et al*. (2009) reported that low levels of CP (< 80 g/kg DM) and high cell wall content, especially lignin have been shown to decrease the digestibility in bush fodders.

However, even when the CP content is above the recommended 80 g/kg DM, Sanon (2007) and Matlebyane *et al*. (2009) reported that most tropical bushes have a high proportion of protein bound to fibre, which could further limit the supply of nitrogen from the bush feed to rumen microbes in order to obtain maximal rate of digestion of the fibre. Other studies also confirmed that cross-linkages of lignin to hemicellulose, polysaccharides and proteins may reduce forage quality through physical toughness and depressed digestibility (Wilson, 1994; Basha *et al*., 2009).

Apart from low CP and high fibre fractions, Reed (1995) reported that condensed tannins present in most bush, could also be responsible for decreasing OM digestibility. The negative effect of tannins on rumen fermentation and OM digestion could be related to the formation of tannin carbohydrate and tannin protein complexes that are less degradable or toxic to rumen microbes (Gemeda and Hassen, 2015).

4.5.3 ***In vitro* methane production**

Reducing enteric methane from ruminants has become a focus of animal nutrition, especially in countries where agriculture is a major economic enterprise because it causes significant losses of energy in the rumen during the utilization process of feed energy (Theart *et al*., 2015). Methane gas production of 92 to 148 ml/g DM from encroacher bushes in this study were higher than those reported by Theart *et al*. (2015), in some Kalahari browse species which ranged between 1.58 and 64 ml/g DM and by Gemeda and Hassen (2015) in some tropical browse plants which ranged from 0.10 to 20.67 ml/g DM. The large variation observed from other reports even for the same species could be associated with the assay method used, species investigated, age of the plant, proportion of leaves to twigs and season of harvest. On the other hand, de Klerk (2016) observed increased methane production from diets with increased fibre contents. This could be also another compounding factor to the high methane production observed in this study given the fact that the browse products used contained high proportions of stem parts, hence high fibre as reported in Chapter 3, especially in the dry season when bushes had few or no leaves.

The reduction of methane production from late dry to early rainy season could be related to their increased phenolic and tannin concentrations observed as season progressed from late dry to early rainy season as reported earlier in Chapter 3. This is in agreement with the observations by Gemeda and Hassen (2015) that browse plants with higher phenolic and tannin contents generally produced lower methane gas regardless of their CP, NDF, ADF, and lignin contents.

Hydrolyzable tannins adversely affect methanogens (Aboagye and Beauchemin, 2019; Goel and Makkar, 2012), but the effect of condensed tannins on the production of methane in the rumen varies. The increased HT concentration from the late dry season to the early rainy season across all species may have contributed to the reduction in methane gas production. Furthermore, there was a reduction in dietary fiber digestibility, which could possibly reduce methane production because H_2 is released during fibrolysis, and it acts as a substrate for methanogenesis in forming acetate from pyruvate (Moss *et al*., 2000; Tavendale *et al*., 2005).

4.6 **Conclusion**

All species considered in this study were relatively low *in vitro* OMD and *in situ* neutral detergent fibre digestibility which constrains them as a sole feed resource for ruminants and may require chemical or enzyme treatments and supplementation to improve their utility. Seasonal variability in fibre and phenolics may affect digestibility and methane production of the four encroacher bush species. Also, it was evident from the results of this study that dietary manipulation is required to improve organic matter and neutral detergent digestibilities while reducing the methane gas production from the species studied.

CHAPTER 5

Nutrients intake, digestibility and nitrogen balance of Damara sheep fed bush-based rations

5.1 Abstract

The study determined the effect of including milled bush from different encroacher bush species as roughage sources in the diets of Damara sheep on intake, digestibility and nitrogen balance. The milled bush used were harvested during the late rainy season and obtained from different farms depending on the region where each species predominantly occurs, as follows: *Senegalia mellifera* and *Dichrostachys cinerea* (Khomas); *Terminalia sericea* (Omaheke) and *Rhigozum trichotomum* (Hardap). Five castrated Damara sheep (37.2 ± 2.4 kg) were used in a 5 x 5 simple change-over design with 5 periods of 21 days each and 5 treatments. Sheep were fed in metabolic cages individually five diets that had roughage included at 40% and differed only by the roughage source, as bush species. A conventional or control diet (T1), consisted of ground lucerne (10%) and grass hay (30%); diets T2-T5 were constituted of milled biomass of the encroacher bush species *S. mellifera*, *D. cinerea*, *T. sericea* and *R. trichotomum*, respectively. All diets were formulated to contain the same crude protein (13%) and energy (9 MJ/kg) content. The intake for dry matter (DM) and organic matter (OM) were affected ($P < 0.0001$) by roughage source. Sheep fed on T5 had higher ($P = 0.007$) ADFom intake and lower apparent digestibility coefficients for DM ($P < 0.001$) than other treatment groups. Diet affected ($P < 0.001$) apparent digestibility of CP; sheep fed T2 and T3 had higher ($P < 0.05$) apparent CP digestibility values compared to those on other diets. The apparent EE, ash, NDFom and ADFom digestibility also differed ($P < 0.05$) among treatments. All treatments had positive nitrogen retention which ranged from 16.2 to 22.1 g/day. The four browse species investigated can be used as roughage sources in Damara sheep diets, provided they are included at 40% of the ration, in combination with other ingredients to support optimal intake, digestibility and nitrogen balance.

5.2 **Introduction**

Sheep production is one of the main socio-economic activities in Namibia, especially in the southern part of the country. However, irregular rainfall and other compounding factors such as bush encroachment make feed availability a great challenge, resulting in low livestock productivity. Natural vegetation including grasses and browse plants, constitute a major component of feed resources for sheep but in most cases especially during the dry season, the grazing is not sufficient to meet their nutritional needs. Browse plants, in particular, provide considerable amount of biomass from leaves and small twigs, flowers and pods during the dry season when other feed resources such as grasses are depleted and are of low nutritional quality (Moleele, 1998; Salem, 2005; Quansah and Makkar, 2012).

In Namibia there is a wide range of browse species that have turned into encroacher bushes and have been progressively taking over millions hectares of farm lands (de Klerk, 2004). Despite their encroaching nature, they still remain potential animal feed resources. Notwithstanding, other utilization strategies such as harvesting and processing edible parts should be encouraged to realize their full potential as fodder in the diets of livestock. The use of milled bush from encroacher bushes has recently been recognised by farmers as roughage to sustain their livestock especially during drought and present a potential valuable feed resource (Honsbein *et al*., 2017).

In addition, Honsbein *et al*. (2017) reported that milled bush is generally characterized by high neutral detergent fibre (NDF) content of >57% and low organic matter digestibility (46 - 48%) which can negatively influence intake. Hence, they further recommended that milled bush requires supplementation with additional concentrates to dilute fibre, improve its nutritional value and digestibility. Another study by Epafras (2019) also reported that *S. melifera* bush-based rations were comparable to grass-based diets on the intake, digestibility and nitrogen balance of Boer goats.

Studies on the digestibility of feed are important as they allow the estimation of nutrients available to the animal. Most of the studies on digestibility of browse fodders used *in vitro* techniques (De Boever *et al*., 1988; Shayo and Ude Ân, 1999; Jančík *et al*., 2008;

Spanghero *et al*., 2010; Baiesi-Ferrchrari *et al*., 2011; Mahesh *et al*., 2017; Cömert Acar, 2018), since they have the advantages of being less costly and less time consuming, good reproducibility and also correlated well with values measured in *in vivo* trials. Nousiainen *et al*. (2003), however, indicated that the *in vivo* digestion trials still remain the more reliable and accurate measure of digestibility because they take into account the biological effects of the animals.

Since the studies by Honsbein *et al.* (2018) and Epafras (2019) were restricted to milled bush from *S. mellifera* on cattle and goats, there is need to explore the feeding value of milled bush from *S. mellifera* and other encroacher bushes as roughage sources in the diets of sheep. Therefore, the objective of this study was to evaluate the effect of inclusion of milled bush from *S. mellifera, D. cinerea*, *T. sericea* and *R. trichotomum* as roughage sources on intake, nutrient digestibility and nitrogen retention by Damara sheep.

5.3 **Materials and Methods**

5.3.1 **Study area**

The feeding experiment was conducted at the Neudamm Campus of the University of Namibia, which is approximately ± 30 kilometres east of Windhoek on the B1 road to Hosea Kutako International Airport, in the Khomas region of Namibia. The campus is situated at 22°30'10.19" S latitude and 17°22'5.39" E longitude.

All procedures conducted during this experiment were approved by the Animal Research Ethics Committee at the University of Namibia (Ethical clearance reference number: AREC/024/2020, shown in Figure 10.1 under Appendix). The experiment was conducted from the 20th November 2019 to the 18th February 2020.

5.3.2 **Harvesting and preparation of bush biomass**

The milled bush biomass of encroacher bush species used in this study was obtained from different farms depending on the region where each species predominantly occurs, as follows: *S. mellifera* and *D. cinerea* (Khomas); *T. sericea* (Omaheke) and *R. trichotomum* (Hardap). Harvesting was done between April and May 2019 and the harvested biomass was restricted to branches or twigs of $\leq$ 20 mm in diameter. The fresh biomass was milled using a hammer mill to a particle size of 10 mm and air dried under shade for two to four days before being packed in bags and transported to Neudamm campus for storage until the feeding trial.

5.3.3 Feed ingredients and experimental diets

Apart from bush biomass, other roughage sources used in the preparation of experimental diets were grass hay (mixed veld grass species) and lucerne hay, which were also milled to particle sizes of 10 mm.

The experimental diets were formulated to constitute 40% of different roughage sources and similar 60% concentrate made from a combination of different feed ingredients (Table 5.1).

The concentrate mix was made from a combination of the following ingredients: yellow maize meal, FeedMaster HPC 30 as a protein concentrate, molasses syrup, Futterfos as a multi-mineral supplement and salt. Except for bush biomass, all other ingredients were purchased from commercial feed supplier.

A conventional diet consisting of coarsely ground lucerne (10%) and grass hay (30%) as roughage, was used as a control (T1), while the other 4 treatment diets (T2-T5) consisted each of the selected bush species as roughage source at the same inclusion rate (40%) and the remaining portion consisted of the same combination of supplements.

All treatment diets were formulated using the Microsoft Excel program, to provide the same amount of protein (iso-nitrogenous) and energy (iso-energetic), containing 13% CP and 9 MJ ME/ kg DM, as well as to meet nutrient requirements of growing sheep according to NRC (2007) recommendations.

The treatment diets used in this experiment were mixed at the experimental site. Except for molasses syrup which was diluted with equal portion of water to make a solution, the rest of the ingredients were mixed on *as is* basis using a concrete mixer.

Table 5.1 Ingredients composition of the five treatment diets

	Treatment diets*				
Feed ingredient (kg as is)	T1	T2	T3	T4	T5
Coarsely ground grass hay	30	0	0	0	0
Coarsely ground lucerne hay	10	0	0	0	0
Milled Bush	0	40	40	40	40
Yellow maize meal	22	20	20	19	20
Molasses syrup	5	5	5	5	5
HPC 30	30	32	32	33	32
Futterfos^{TM} P14	1.5	1.5	1.5	1.5	1.5
Coarse salt	1.5	1.5	1.5	1.5	1.5
Total	100	100	100	100	100

* T1- control diet; T2– *Senegalia mellifera*-based diet; T3 - *Dichrostachys cinerea*-based diet; T4- *Terminalia sericea*-based diet and T5 - *Rhigozum trichotomum*-based diet, HPC 30-high protein concentrate; Futtterfos^{TM} - P14- Phosphate lick with 14% Phosphorus

5.3.4 **Experimental animals and management**

Five castrated 13 month old Damara sheep, with an average initial body weight of 37.2 ± 2.4 kg were used in this study. The sheep were housed in individual metabolism cages (120 cm long, 54 cm wide and 90 cm above the ground) fitted with troughs for feed and water, that allowed the total collection of faeces and urine separately. The urine was collected on a sheet metal chute from the base of the metabolism cages and directed via urine collection plates into the bottles.

The sheep were vaccinated with injectable Multivax P™ (Intervet South Africa (Pty) Ltd) at the beginning of the study to protect them against botulism, black quarter, pulpy kidney and clostridium. They were also injected with Dectomax™ (Pfizer Laboratories (Pty) Ltd, Sandton) against internal and external parasites.

They received treatment diets and drinking water on an *ad lib* basis. After the adaptation period, the feed offered and feed refused daily were weighed using a 7.5 kg x 0.1g Digital table top weighing scale and recorded to determine the feed intake. The amount of daily feed offered to sheep was then adjusted daily and calculated at a 15% refusal level. The daily feed was offered to each sheep in two portions, namely at 09h00 and 14h00.

5.3.5 Experimental design

The study used a 5 x 5 simple change-over design with five changeover periods of 21 days, resulting in total of 105 days for the whole feeding experiment. Each 21 days period, comprised of 14 days for the sheep to adapt to the diets and experimental environment, and seven days for daily collection of feed, refusals, faeces and urine. Sheep were weighed before the beginning of each period and changed treatment diets randomly, allowing all sheep exposure to each treatment diet.

5.3.6 Feed, refusal, faeces and urine collection

During the 7 days of each change over period (days 15-21), a random sample of each treatment diet was taken every day from a daily feed portion and pooled separately in marked paper bags.

The daily refusals, faeces and urine output for each sheep were also collected every morning before feeding, at 9:00 and immediately measured, during each change over period (days 15-21). Urine was collected in containers containing 25 ml of 10% sulphuric acid to prevent loss of nitrogen by volatilization (Mlambo *et al*., 2004). A sample of 10% daily faecal and urine output was taken and stored at -4°C, pending chemical analysis. The frozen daily urine samples were later thawed, pooled for each animal for the 7 d period and sub-sampled a composite sample for N analysis. Similarly the daily frozen faecal samples were later thawed, dried at 60 °C to constant weight and bulked per animal over the collection period of 7 days, sub-sampled a composite sample and then milled to pass a 1-mm sieve prior to chemical analyses. At the end of each period, the refusals for each sheep were mixed and pooled. Representative samples were taken, ground to pass a 1 mm sieve and stored in plastic bottles for chemical analysis.

5.3.7 Chemical analysis

Samples of feed offered, refusals and faeces were analysed for different constituents as per the methods described below and the results used to estimate intake, digestibility and retention of these constituents.

The detailed procedures on chemical analysis and determination of dry matter (DM), Ash, crude protein (CP), Ether extract (EE), Ash-free neutral detergent fibre (NDFom), Ash-free acid detergent fibre (ADFom), Ash-free Acid detergent lignin (ADLom), Hemicellulose and Organic matter (OM, are outlined in Section 3.3.4 of Chapter 3.

Urine samples were analysed for total nitrogen using the crude protein (CP) method no. 978.04 (AOAC, 2005).

5.3.8 **Calculations**

The apparent digestibility of feed nutrients was calculated as per the formulae used by Furtado *et al*. (2020):

$$\text{Digestibility coefficient of the DM} = \frac{\text{DM intake (g/d) - Faecal DM (g/d)}}{\text{DM intake (g/d)}}$$

Using the same procedure (by replacing DM with other nutritional constituents, such as CP, EE, etc.), the digestibility coefficients of OM, CP, EE, NDFom, ADFom and N were calculated.

Nitrogen retention was calculated as the difference between N intake and losses through faeces and urine (Mlambo *et al*., 2004; Furtado *et al*., 2020), as follows:

N-balance (g/d) = Intake N (g/d)-faecal N (g/d)-urine N (g/d)

5.3.9 **Statistical analysis**

The experiment design was a simple change-over design with 5 treatments and 5 subjects (sheep) over 5 periods. The data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (2009), with the main effect being the treatments and the following statistical model was used:

$y_{ijk} = \mu + A_i + P_j + \mathrm{T}_k + \varepsilon_{ijk}$

Where:

y_{ijk} = response variable of interest on animal *i* in period j under treatment k;

μ = the overall mean;

A_i = the effect of i^{th} the animal;

P_j = the effect of j^{th} period;

T_k = effect of the k^{th} treatment;

ε_{ijk} = random error

Means were separated using the Turkeys' Studentised range test. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences. In addition, differences among means with $0.05 \leq P \leq 0.10$ were accepted as representing tendencies to differences.

5.4 **Results**

5.4.1 **Chemical composition of treatment diets**

The chemical compositions of the treatment diets used in this study are shown in Table 5.2. Dry matter (DM), organic matter (OM), ether extracts (EE), neutral detergent fibre (NDFom) and acid detergent fibre (ADFom) of the treatment diets were not influenced ($P > 0.05$) by the roughage source. There was a trend ($P = 0.073$) for the CP to differ among the bush-based diets.The ADLom differed ($P = 0.001$) among diets; ADLom for control diet T1 was lower ($P < 0.05$) than other treatments. Treatment T2 had lower ($P < 0.05$) ADLom than T3 and T4, but was similar ($P > 0.05$) to T5.

Table 5.2 Chemical composition (g/kg DM) of five treatment diets

	Treatment diets*						
	T1	T2	T3	T4	T5	SEM	P-value
Dry matter (DM)	929.3	924.1	925.6	926.3	932.3	6.6	0.910
Ash	114.9^{a}	108^{ab}	99.8^{b}	106.7^{ab}	95.4^{b}	4.4	0.042
Organic matter (OM)	814.4	816.1	825.8	819.6	836.9	8.8	0.397
Crude protein (CP)	141.6	131.9	132.6	122.7	123.9	4.8	0.073
Ether extract (EE)	18.1	17.2	16.6	18.8	17.8	1.5	0.889
Neutral detergent fibre (NDFom)	405.1	414.0	425.3	415.1	434.5	19.7	0.854
Acid detergent fibre (ADFom)	224.7	237.5	274.5	288.2	288.0	23.7	0.224
Acid detergent lignin (ADLom)	44.3^{c}	73.3^{b}	93.3^{a}	93.3^{a}	84.1^{ab}	3.6	0.001
Metabolizable energy (estimated MJ/kg)[1]	9.3	9.9	9.6	9.1	9.8	N/A	N/A

* T1 = control diet; T2 = *Senegalia mellifera*-based diet; T3 = *Dichrostachys cinerea*-based diet; T4 = *Terminalia sericea*-based diet; and T5 = *Rhigozum trichotomum*-based diet

[1] Estimated metabolizable energy (ME) values based on the feed formulation program; SEM = Standard error of means**;** $^{a-c}$Means with different superscripts within the same row differ significantly **(**$P < 0.05$**)**

The ash content differed ($P = 0.042$) among the diets. The control diet T1 had higher ($P < 0.05$) ash content than T3 and T5, but did not differ from treatments T2 and T4. The ash content for T2 tended ($P = 0.054$) to be higher than T5, but was similar ($P > 0.05$) to other treatments. The estimated metabolizable energy (ME) based on the feed formulation program ranged between 9.1 and 9.9 MJ/kg among the treatment diets.

5.4.2 **Nutrient intake and apparent digestibility**

The daily intakes and apparent digestibility of nutrient constituents by Damara sheep fed treatment diets are presented in Table 5.3. The dry matter (DM) intake differed ($P < 0.0001$) among diets. Intake of DM for T1 was greater ($P < 0.05$) than for other treatments. Among the bush-based diets, DM intake of T4 and T5 was similar ($P > 0.05$), but higher ($P < 0.05$) than T2 and T3. The organic matter (OM) intake was similar among the treatment diets, except for T5 which was higher ($P < 0.05$) than the other four treatments.

Table 5.3 Daily intakes and apparent digestibility coefficients of nutrient constituents by Damara sheep on different diets

	Treatment diets*						
Nutrient constituents	T1	T2	T3	T4	T5	SEM	P-value
Intake (g DM /day)							
DM	1796.7^{a}	1689.7^{c}	1699.2^{c}	1765.6^{b}	1769.8^{b}	8.2	<0.0001
OM	1336.4^{b}	1311.2^{b}	1342.6^{b}	1336.6^{b}	1397.7^{a}	13.7	0.0016
CP	253.3^{a}	233.0^{b}	229.6^{b}	220.8^{b}	223.4^{b}	4.5	<0.0001
Ether extract (EE)	32.2	30.5	28.8	33.6	32.2	1.6	0.2903
NDFom	710.0	688.7	652	718.2	768.8	26.5	0.0524
ADFom	394.2^{b}	377.7^{b}	398.0^{b}	493.0^{a}	505.2^{a}	29.6	0.0069
Digestibility coefficiencies							
DM	0.720^{a}	0.724^{a}	0.726^{a}	0.708^{b}	0.676^{c}	0.004	<0.0001
OM	0.698^{b}	0.711^{a}	0.712^{a}	0.683^{c}	0.660^{d}	0.004	<0.0001
CP	0.787^{bc}	0.801^{b}	0.829^{a}	0.762^{d}	0.776^{cd}	0.007	<0.0001

Ether extract (EE)	0.764^{ab}	0.768^{ab}	0.805^{a}	0.819^{a}	0.694^{b}	0.030	0.0451
NDFom	0.634^{a}	0.592^{b}	0.549^{c}	0.592^{b}	0.540^{c}	0.014	0.0003
ADFom	0.584^{a}	0.510^{ab}	0.368^{c}	0.457^{b}	0.416^{bc}	0.027	<0.0001

* T1 = control diet; T2 = *Senegalia mellifera*-based diet; T3 = *Dichrostachys cinerea*-based diet; T4 = *Terminalia sericea*-based diet; T5 = *Rhigozum trichotomum*-based diet; SEM = Standard error of means**;**

$^{a-c}$Means with different superscripts within the same row differ significantly **(**$P < 0.05$**)**

The CP intake was higher for T1 ($P > 0.05$) compared to other treatment groups; CP intake for T2 tended ($P = 0.07$) to be higher than T4. The ether extract (EE) intake was similar ($P > 0.05$) among all treatment diets. The intake of NDFom was higher ($P = 0.05$) for T5 than T2 and T3. On the other hand, ADFom intakes for T4 and T5 were similar ($P > 0.05$), but higher ($P < 0.05$) than the other three treatment groups. Ash intake for diets T3 and T5 were similar ($P > 0.05$), but lower ($P < 0.05$) than the other three treatment groups. Ash intake for T1 tended ($P = 0.08$) to be higher than T2.

The apparent digestibility of all nutrients was affected ($P < 0.05$) by the source of roughage. Treatment T5 had lower digestibility ($P < 0.05$) for DM and OM compared to the other treatment groups. The CP digestibility for T3 was higher ($P < 0.05$) than the rest; CP digestibility for T2 was higher (< 0.05) than that for T4 and T5, but was similar ($P > 0.05$) to that for T1. The fibre fractions (NDFom and ADFom) were more digestible ($P < 0.05$) for T1 than the bush-based diets (T2-T5), while among the bush-based diets, T3 and T5 had lower ($P < 0.05$) digestibility coefficients for fibre fractions than others. The ash digestibility coefficients were higher ($P < 0.05$) for T3 than other diets.

5.4.3 **Nitrogen balance**

The nitrogen (N) utilization parameters by Damara sheep were highly ($P < 0.0001$) influenced by the roughage source (Table 5.4). The nitrogen intake was similar ($P > 0.05$) amongst the bush-based treatment groups but lower ($P < 0.05$) than the control group (T1). Faecal N in T3 was lower ($P < 0.05$) than for other diets. Faecal N for T1 was similar ($P > 0.05$) to T4, but higher ($P < 0.05$) than for T2 and T3. Urinary N losses were higher (P

< 0.05) in sheep offered T5 than in other treatment groups; however, sheep on T4 had lower ($P < 0.05$) urinary N than other treatments. Urinary N excretion was similar ($P > 0.05$) for T1, T2 and T3.

Table 5.4 Nitrogen utilisation of Damara sheep fed different diets

	Treatment diets*						
	T1	T2	T3	T4	T5	SEM	P-value
N_intake (g DM/day)	40.3^a	37.3^b	36.7^b	35.3^b	35.7^b	0.70	<0.0001
Feacal_N (g DM/day)	8.6^a	7.4^b	6.2^c	8.4^a	8.0^a	0.23	<0.0001
Urine_N (g /day)	9.6^b	9.3^b	9.0^b	6.9^c	11.6^a	0.43	<0.0001
N-digestibility coefficient	0.787^{bc}	0.800^b	0.829^a	0.762^d	0.776^{cd}	0.01	<0.0001
N-retention (g /day)	22.1^a	20.6^a	21.5^a	20.1^a	16.2^b	0.74	<0.0001
N-retention (% N intake)	55.0^a	54.6^a	58.2^a	56.9^a	45.5^b	1.28	<0.0001

* T1 = control diet; T2 = *Senegalia mellifera*-based diet; T3 = *Dichrostachys cinerea*-based diet; T4 = *Terminalia sericea*-based diet; and T5 = *Rhigozum trichotomum*-based diet; SEM = Standard error of means**;**

$^{a-d}$Means with different superscripts within the same row differ significantly **(**P < 0.05**)**

The N digestibility coefficient ranged from 0.762 to 0.829. Sheep on T3 had higher (P < 0.05) N digestibility than those on other diets. The N digestibility of sheep on diet T2 was similar (P > 0.05) to T3, but was higher (P < 0.05) than those on the rest of the diets. Overall, all consumed diets recorded a positive N-retention, with only T5 being lower (P < 0.05) than the other treatment groups, which showed similar (P > 0.05) N-retention.

5.5 Discussion

5.5.1 Chemical composition of dietary treatments

The five treatment diets were formulated to provide the same amount of crude protein (iso-nitrogenous) and energy (iso-energetic) i.e. 13% CP and 9 MJ/kg ME, as calculated from the nutritional contents of different ingredients used in the diets. It is evident, however, from Table 5.2 that the analysed CP contents of the treatment diets deviated slightly from the calculated values (13%) with the control diet T1 being higher by 1% while T4 and T5 were slightly lower by 0.7%. The variation in the CP content could be due to variation in the CP contents of different batches of roughage sources used in the respective diets. This could be due to the grass bales used being cut from different areas and composed of different grass species, hence not of the same quality, even though they were obtained from the same feed supplier. Similarly, although the milled bushes were obtained from the same supplier or farm, it would be practically impossible to have the same quality milled bush in each packed bag because of the differences in the proportion of branches to leaves making up a batch. Hence some nutrient variation between the formulated and actual values (Table 5.2) could be expected. Notwithstanding, the CP levels in all treatment diets were within the margin of error of the minimum 13% requirement for growth in ruminant as indicated by NRC (2007).

In this study, the NDFom contents of the diets were not significantly different even though they were from different sources and they were also above the concentration of 60 - 65%, which is suggested to limit intake and digestibility of nutrients in ruminants (Van Soest *et al.*, 1991a). On the other hand, the significant variation in the ADLom and ash concentration of the treatment diets could be an indication of variability in species, soil, climate and the growth phenology of the bush and grass species due to sampling from different agroecological zones. This is consistent with Smith (2008) who reported that roughage source may also contribute to nutrient variation in animal diets as there were large differences found in nutrient densities within the same roughage source due to various factors such as locality, climate, soil and production practices.

5.5.2 **Nutrient intake and apparent digestibility**

In this study, different roughage sources were used to provide fibre as required in ruminant nutrition. However, Mertens (2002) cautioned against feeding an excessive amount of fibre to the animal because it can increase rumen fill and reduce DM intake and digestibility, hence growth. Galyean and Hubbert (2014) also emphasized that both source and level of roughage affect DM intake. In this case, the inclusion level of roughage was the same in all diets but different sources of roughage used, seem to have influenced both the DM intake and digestibility. The replacement of grass hay with milled bush, irrespective of the species, significantly reduced DM intake. Higher NDF digestibility is associated with increased DM intake (Oba and Allen, 1999) and this explains the reduced intake of the bush-based diets. Furthermore, the bush-based diets had higher ADLom and lower CP compared to T1, contributing to reduced intake (Detmann et al., 2014; Adesogan *et al.*, 2019).

Among the bush-based diets, it appears that roughage from certain bush species, particularly *S. mellifera* and *D. cinerea* used in T2 and T3, respectively, had more depressing effects on DM intake than other species such as *T. sericea* (T4) and *R. trichotomum* (T5). This could be associated with the differences in the physical structure or characteristics of roughage sources such as coarseness, bulkiness, and abrasiveness, also reported by Suárez *et al*. (2007) and Galyean and Hubbert (2014). Probably, these observations were also due to differences in some properties between grasses and woody fractions of bush species such as a greater flow rate from the rumen for the grass-based diet observed by Trulla (2013), similar to our results of T1 compared to bush-based diets (T2-T5).

In line with this, it was observed that sheep fed on T2 and T3 showed a high tendency to selecting and sorting their feed; they selected the concentrate mix, followed by small particles of roughage and left the large particles. Even though all roughage sources were intended to be ground to the same particle size, the physical structure of the roughage used in diet T2 and T3 could not permit blending in well with the concentrate mix as was the case with the other three treatment diets (T1, T4 and T5).

Sheep fed the control diet T1 had significantly higher CP intake than those fed bush-based diets (T2-T5), which may be linked to the tendency for a higher CP concentration in T1 compared to the other diets. Additionally, the sheep fed T1 also had high DM intake than other treatment groups, which could have contributed to their increased CP intake, consistent with the observation by Furtado *et al*. (2020) of a high correlation between DM and CP intake.

The relatively high apparent digestibilities for DM, OM, CP and EE indicate the ability of Damara sheep to utilize these diets. Nonetheless, the apparent digestibility values for DM and OM by sheep fed T4 and T5 were lower than the other diets (T1 – T3) due to high ADFom and ADLom contents, which have been reported to reduce digestibility in bush fodder (Matlebyane *et al*., 2009; Grabber *et al*., 2009). Although the ADLom for diets T3 and T4 were similar, apparent digestibilities of NDFom and ADFom for T4 were higher, which could be attributed to differences in degree of cross linking of lignin among cell wall components (Grabber *et al.*, 2009; Raffrenato *et al*., 2017). On the contrary, the higher apparent digestibilities of NDFom and ADFom for T1 and T2 relative to T3-T5, are due to the lower ADLom in the former. Besides lignification, apparent digestibility is also influenced by diet composition, which influences activity of fibrolytic microorganisms and the rate at which particles pass through the rumen (Nozière *et al*., 2010).

In contrast to the *in vitro* results (Table 4.1, Chapter 4), where the digestibilities of the browse species ranged from 294.7 to 424.4 g/kg OM during the early rainy season, the *in vivo* digestibilities were drastically improved in the formulated diets (Table 5.3). The improvement is attributed to dilution of the fibre in the browse species and to the provision of energy, supplemental nitrogen and other nutrients to support rumen microbial activity. This supports the current practice in Namibia where livestock farmers use browse in formulating diets for ruminants (Anton Dresselhaus, pers. communication).

5.5.3 **Nitrogen balance**

In agreement with other studies, urinary N was strongly correlated with N intake (Fanchone *et al*., 2013). Crude protein is broken down to ammonia by rumen microbes and the excess ammonia which is not recycled is absorbed in the blood stream and the liver converts it to urea for excretion in urine (Mui *et al*., 2002). The lower faecal N excreted by sheep fed T3 diet could be as a result of its high apparent N digestibility than the other four diets, resulting in its better utilization. Diets T2 and T5 differed in N retention despite *R. trichotomum* and *S. mellifera*, the respective basal bush materials having similar soluble protein (fractions A + B1) (Table 3.2), required for rumen microbial functioning. This could be attributed to lower readily available fermentable energy in T5. The high urinary N excreted for T5 may be attributed to the low apparent NDFom and ADFom digestibilities, resulting in reduced energy supply, hence excess rumen-degradable N was excreted in urine (Nocek and Russell, 1988).

On the other hand, the significant low urinary N from sheep fed T4 could probably be linked to the high extractable tannins observed in *T. sericea* used in T4 especially during the rainy season in which the bush biomass were harvested (Table 3.3 under Chapter 3). Mlambo *et al*. (2004) indicated that some tannins are known to cause improved N balance through the formation of indigestible complexes with protein, which could escape ruminal digestion and only gets digested and utilised in the lower part of the digestive tract, thus acting as a by-pass protein source. This process is said to have beneficial effects by reducing the excess ammonia production in the rumen which results in decreased urinary N excretions. Furthermore, consumption of shrubs containing tannins has been reported to shift excretion of N from urine to feaces in ruminants (Hagerman *et al*., 1992; Kaitho, 1997). Diet T5 led to a greater excretion of N in feaces than urine, which is consistent with its higher condensed tannin (CT) concentration compared to the other browse species (Table 3.3 under Chapter 3).

All treatment diets had a positive and similar N retention, except sheep fed the T5 diet retained significantly lower N, even when expressed as a proportion of N intake, compared to the other four diets. The positive N balance on all the diets indicates the potential of bush-based feeds in facilitating N utilization, provided a readily available source of fermentable energy (for example crushed maize) is included.

5.6 Conclusion

This study showed that intakes of DM were within the ranges reported for sheep and digestibility coefficients of > 0.70 could still be obtained by replacing grass hay with milled bush from encroacher species at inclusion level of 40% in mixed diets of sheep. The results of positive nitrogen retention of > 45% of N intake, showed that the combination of the milled bush and other supplements resulted in palatable diets and met the requirements of the Damara sheep for nitrogenous compounds. Therefore, the use of browse plant resources as roughage, should be given adequate consideration in ruminant production to achieve better productive performance, especially in dry seasons or drought periods.

CHAPTER 6

Feed intake and growth performance of Damara lambs fed bush-based rations from four Namibian encroacher bush species

6.1 Abstract

A study was conducted to determine the effect of feeding bush-based finishing rations on the intake and growth performance of Damara lambs. Thirty Damara Lambs (15 males and 15 females, on average 5 months old, average initial weight of 16.7 ± 1.9 kg) were allocated to five treatments in a completely randomized experimental design over a 90-day feeding study. A conventional diet was used as a control (T1), consisting of coarsely ground Lucerne (10%) and grass hay (30%) as roughage. Four treatment diets (T2-T5) consisted each of the selected bush species: *Senegalia mellifera*, *Dichrostachys cinerea*, *Terminalia sericea* and *Rhigozum trichotomum*, (40%) and concentrate mix (60%). The average daily feed intake (ADFI) of the lambs (kg DM/kg $W^{0.75}$per day) was affected ($P < 0.05$) by sex, treatment, week and treatment x week interactions. The ADFI for T1 exceeded ($P < 0.05$) that for T2 and T3, at most time points. The ADFI of T1 and T4 were similar ($P > 0.05$) at weeks 6 to 10, but differed ($P < 0.05$) at other time points. The ADFI of diets T2 and T3 were similar ($P > 0.05$) except at week 8. Intake of diet T5 exceeded ($P < 0.05$) that of T3 from weeks 4 to 11. Body weights were influenced ($P < 0.05$) by week, treatment x week and treatment x sex interactions. The body weights increased linearly, but with fluctuations by diet. Pairwise contrasts of body weights for sheep on different diets across the weeks were not significant ($P > 0.05$). The average daily gains (ADG) and feed conversion ratio (FCR) were affected ($P < 0.05$) by sex and treatment. T2 had a lower ($P < 0.05$) FCR compared to other bush-based treatment groups (T3, T4 and T5), but a trend ($P = 0.098$) of a better FCR was observed when T2 was compared to T1. The FCR for T3, T4 and T5 were also similar ($P > 0.05$) to the control group T1. Bush-based diets can serve as production diets for weaned sheep without adversely affecting weight gain.

6.2 Introduction

In most tropical livestock production systems, sheep are raised under rangeland conditions, predominantly without any feed supplement. A major constraint to such a livestock production system is the scarcity and fluctuating quantity and quality of the year-round feed supply (Olafadehan and Adewumi, 2010). Under such unfavourable conditions, animals have low productivity and as a consequence, it takes longer for animals to reach slaughter weights and often produce lower quality carcasses (Ben Salem *et al.*, 2004). The situation becomes critical during the long dry season and drought years when there is little or no available standing hay forages.

As an alternative, finishing sheep in the feedlot can play an essential role in preparing lambs for slaughter, as well as relieving the grazing pressure on pasture. Roughage is a major component of the feedlot diets as over 75 % of small, medium and large scale operations supply grain and hay (or a roughage source) on *ad libitum* basis (Jolly and Wallace, 2007). Mertens (2002) reported that a minimum amount of roughage included in the feedlot diets is necessary to maintain a healthy digestive system. The chop length and the type of fibre used determine the rate at which the fibre is degraded in the rumen, and are seen as the most important factors relating to the fibre requirement of the diet. Ideally, 20 % roughage in the ration should be sufficient to maintain rumen pH and a minimum of 27-30 % NDF (Mertens, 2002).

Mutton and lamb producers especially in Namibia and South Africa use lucerne and grass hay as roughage source in feedlot diets (van der Merwe *et al.*, 2020). However, the low availability and high cost of these ingredients in the market often limit its use as roughage in feedlot diets. Hence, interventions to find affordable and sustainable alternative roughage sources are important for the sheep industry and the environment. These will enable livestock to survive critical periods of feed shortages and still be able to produce good quality meat.

In Namibia, there is an abundance of encroaching bushes that present an opportunity to be explored as a possible alternative feed resource for livestock. Honsbein *et al.* (2018) evaluated the feeding value of *S. mellifera* milled bush as a replacement for grass hay in total mixed feedlot diets of Sanga cattle. The study concluded that bush-based diets can be used for growing finishing Sanga steers in the feedlot, which achieved the average feed intake of 7 kg DM/day/head and average daily gain of 880 g/day. To our knowledge, there is paucity of information on the feeding value of other encroacher species, as alternative fodders to the conventional feed like grass and lucerne hay for ruminant livestock. In addition, it is also necessary to test performance of other ruminant species like sheep when finished on feedlot diets containing milled bush as a roughage source.

It was hypothesized that milled encroacher bushes such as *S. mellifera*, *D. cinerea*, *T. sericea* and *R. trichotomum* could be used as a suitable alternative roughage source to grass hay, for feedlot animals and may improve the overall performance of sheep. Therefore, the objective of this study was to evaluate the effects of feeding the four encroacher bush species as alternative roughage to grass hay (conventional fodder) on the feed intake and growth performance of Damara sheep.

6.3 Materials and Methods

6.3.1 Study area

The feeding experiment was conducted at the same study areas, under the same ethical clearance approval as described in Section 5.3.1 of Chapter 5. The experiment was conducted from October 2019 to January 2020.

6.3.2 Experimental animals and management

All lambs used belonged to the University of Namibia, Neudamm campus farm. Thirty (30) weaned 5 month old Damara sheep lambs (15 males and 15 females) with a mean weaning weight (WW) of 16.74 ± 1.9 kg were used. Sheep were vaccinated with Multivax P™ (Intervet International B.V., The Netherlands) at the beginning of the trial to protect them against botulism, black quarter, pulpy kidney and clostridium. Sheep were also treated with Dectomax™ (Pfizer Laboratories (Pty) Ltd, Sandton) against internal and external parasites.

6.3.3 Feed ingredients and experimental diets

This is described under Section 5.3.2 and 5.3.3 for Chapter 5, as per the ingredients composition of the experimental diets presented in Table 5.1

6.3.4 **Experimental design**

The experiment was set up as a completely randomized design (CRD) with six (6) lambs per treatment (3 males and 3 females), to evaluate feed intake and the growth performance for 90 days, after an adaptation period of 14 days. The 30 lambs were randomly and equally allocated to the treatments. The lambs were housed individually in pens of 1 m^2 with concrete floors in an open-sided roofed shed, where they received treatment diets and water on an *ad lib* basis. Considerations were made during the random allocation of the lambs in respective pens, not to place all animals on the same treatment next to each other in order to minimize the probability that a specific treatment may be affected either positively or negatively by environmental factors or any incident due to pen location and/or conditions.

6.3.5 **Feed Intake**

On arrival at the experimental site, the lambs were given an adaptation period of 14 days, during which they were gradually introduced to the different treatment diets, so as to adapt the rumen microbiota to the new diet. This pretest adjustment period also allowed them to become accustomed to the *ad libitum* feeding routine and adapt to the housing facility.

After the adaptation period, the feed offered and feed refused daily were weighed using a 7.5 kg x 0.1g Digital table top weighing scale and recorded to determine the feed intake. The amount of daily feed offered to sheep was then adjusted at the beginning of every week and calculated at a 15% refusal level. The daily feed was offered to each sheep in two portions, namely at 09h00 and 14h00. Feed spilled on the floor by sheep in each pen during feeding were picked up during the next feeding session and collected in plastic bags as part of feed refused. Every morning before feeding, fresh clean water was provided to each lamb daily in their individual buckets. The water buckets were refilled during the next feeding session and at 17h00. The water buckets were brushed and cleaned once a week.

6.3.6 **Body weight**

Initial body weights were obtained by weighing the lambs at the beginning of the adaptation period. Thereafter, the lambs were weighed weekly on the same day of the week in the morning before feeding, until the end of the experiment. The calibrated electronic scale (Micro T7E Scale; Premier Scale Services (Pty) Ltd) was used to weigh lambs throughout the experiment period. Average daily gains were calculated as differences between final and initial body weights divided by number of feeding days. The feed conversion rations were also calculated by dividing the total feed intake over total weight gain during the feeding period.

6.3.7 Sampling and chemical composition analysis of experimental diets

During the experiment period, random samples of each treatment diet were taken once from a weekly batch, mixed and pooled separately in marked paper bags. At the end of the experiment period, all treatment diet samples were ground to pass a 1 mm sieve (Retsch Mable mill; Retsch GmbH) and stored in plastic bottles for chemical analysis. The detailed procedures for chemical composition analyses are outlined in Section 3.3.4 of Chapter 3.

6.3.8 Statistical Analysis

Intake is a function of the body weight (W) of the animal, hence analyses were based on metabolic body weight ($W^{0.75}$). The model (1) included the effects: sex, treatment, week and treatment x week interactions. Data was analysed using Proc Mixed (SAS, 2009) which takes into account correlation between repeated measures on an individual and the Bayesian Information Criterion (BIC) which compares covariance structures based on goodness of fitness criteria, was used to select the appropriate covariance structure (Littell *et al.*, 1998), which was simple. Estimate statements were used in Proc Mixed (SAS, 2009) to compare means and obtain standard errors. Effects were considered significant at $P < 0.05$. Trends were declared when $0.05 \leq P \leq 0.10$.

$$Y_{ijkl} = \mu + F_i + S_j + T_k + FT_{ik} + e_{ijkl} \qquad (1)$$

Where:

Y_{ijkl} = feed intake

μ = overall mean

F_i = effect of treatment (i = T1, T2, T3, T4 and T5)

S_j = effect of sex (j = m, f)

T_k = effect of week (k =1,2,3…13)

FT_{ik} = interaction effect between treatment and week

e_{ijkl} = random error term explaining variation among experimental units (EU) on the same treatment.

A similar model to (1) was used to fit body weights, but with the additional interaction effect of treatment by sex. The treatment and sex effects were not significant ($P > 0.05$), but were included in the model because the treatment x week and treatment x sex interactions were significant ($P < 0.05$). The best covariance structure for body weights was ante-dependence [ANTE(1)].

$$Y_{ijkl} = \mu + F_i + S_j + T_k + FT_{ik} + TS_{jk} + e_{ijkl} \qquad (2)$$

Where:

Y_{ijkl} = body weight

μ = overall mean

F_i = effect of treatment (i = T1, T2, T3, T4 and T5)

S_j = effect of sex (j = m, f)

T_k = effect of week (k = 1,2,3…13)

FT_{ik} = interaction effect of treatment and week

FS_{jk} = interaction effect between treatment and sex

e_{ijkl} = random error term explaining variation among experimental units (EU) on the same treatment.

The chemical composition of the diets was subjected to analysis of variance (ANOVA) using Proc GLM (SAS, 2008). Similarly, FCR and ADG were subjected to analysis of variance (ANOVA) using Proc GLM (SAS, 2008) with the effects in the model being sex and treatment.

6.4 Results

6.4.1. Chemical composition of treatment diets

The chemical composition of the treatment diets used in this study is shown in Table 6.1. The *D. cinerea*-based diet (T3) had the highest dry matter (DM) and organic matter (OM) contents ($P < 0.0001$), while *T. sericea*-based diet (T4) had the lowest. The crude protein (CP) for the control diet (T1) was similar to the *S. mellifera*-based diet but higher ($P = 0.0270$) than T3, T4 and T5, which did not differ ($P > 0.05$). The neutral detergent fibre (NDFom) and acid detergent fibre (ADFom) contents for T3 were higher ($P < 0.0001$) than those of the other four treatment diets. The control diet, however, had the lowest ADF, while T4 and T5 had the lowest NDF content. The control diet also had the lowest ($P < 0.0001$) acid detergent lignin (ADLom) content compared to the bush-based diets.

The ether extract (EE) content was higher ($P < .001$) in T3 and T4 among the bush-based diets, but the control diet had the lowest content amongst all the treatment diets. The ash content was highest ($P < 0.0001$) for T4 amongst the bush-based diets, but was similar to the control diet. The calcium content differed ($P = 0.0006$) among treatments; T4 and T2 had higher concentration, than T1, T3 and T5 which were similar ($P > 0.05$).

Table 6.1 Chemical composition (g/kg DM) of five treatment diets

	Treatment diets*						
Variables	T1	T2	T3	T4	T5	SEM	P-value
Dry matter (DM)	910^{b}	903^{c}	918^{a}	890^{d}	904^{c}	1.04	<0.0001
Organic matter (OM)	869^{c}	890^{b}	904^{a}	869^{d}	885^{b}	1.07	<0.0001
Crude protein (CP)	146^{a}	143^{ab}	135^{c}	140^{bc}	139^{bc}	1.53	0.0270
Neutral detergent fibre (NDFom)	415^{b}	422^{b}	506^{a}	386^{c}	386^{c}	5.12	<0.0001
Acid detergent fibre (ADFom)	221^{d}	226^{b}	365^{a}	245^{c}	240^{c}	3.37	<0.0001
Acid detergent lignin (ADLom)	44.3^{c}	73.3^{b}	93.3^{a}	93.3^{a}	84.1^{ab}	3.55	0.0009
Ether extract (EE)	16.0^{d}	16.8^{c}	19.3^{a}	20.0^{a}	17.5^{b}	0.16	<0.0001
Ash	131^{a}	110^{c}	96^{d}	131^{a}	115^{b}	1.07	<0.0001
Calcium (Ca)	9.3^{c}	11.5^{b}	9.6^{c}	13.2^{a}	9.7^{c}	0.27	0.0006
Phosphorus (P)	0.507^{c}	0.475^{d}	0.410^{e}	0.570^{a}	0.530^{b}	0.002	<0.0001
Metabolizable energy (MJ/kg)[1]	9.3	10.0	9.6	8.9	9.8	N/A	N/A

* T1 = control diet; T2 = *Senegalia mellifera*-based diet; T3 = *Dichrostachys cinerea*-based diet; T4 = *Terminalia sericea*-based diet; and T5 = *Rhigozum trichotomum*-based diet

[1] Estimated metabolizable energy (ME) values based on the feed formulation program; SEM = Standard error of means; $^{a-d}$Means with different superscripts within a row differ significantly (P< 0.05)

6.4.1 **Feed intake**

The average daily feed intake (ADFI) of the sheep (kg DM/kg $W^{0.75}$per day) during the ninety (90) days feeding period was affected ($P < .05$) by sex, treatment, week and treatment * week interaction (Figure 6.1).

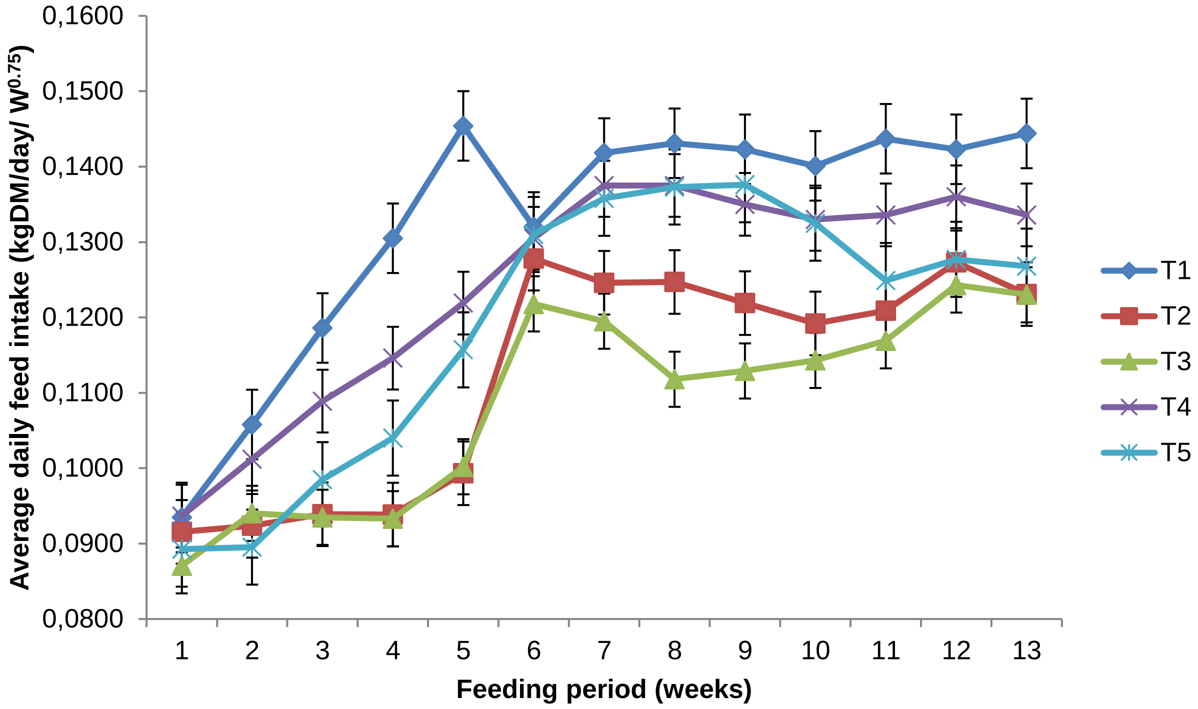

Figure 6.1 The average daily feed intake (kg DM/kg $W^{.75}$ per day) of weaned Damara sheep

* T1 = control diet; T2 = *Senegalia mellifera*-based diet; T3 = *Dichrostachys cinerea*-based diet; T4 = *Terminalia sericea*-based diet; and T5 = *Rhigozum trichotomum*-based diet

The ADFI generally increased from week 1 to week 6 - 7 and then stabilized, although lowest intake was for T2 and T3, which also showed the greatest lag. Average daily feed intake for the control diet T1 exceeded ($P < 0.05$) that for T2 and T3, at most time points. Other than at weeks 3, 4, 5, 11 and 13, ADFI for T1 and T4 did not differ ($P > 0.05$). The ADFI of T1 and T4 were similar ($P > 0.05$) at weeks 6 to 10, but differed ($P < 0.05$) at other time points. The ADFI of diets T2 and T3 were similar ($P > 0.05$) except at week 8. Intake of diet T5 exceeded ($P < 0.05$) that of T3 from weeks 4 to 11.

From estimated contrasts, the ADFI of the control diet appears to have peaked at week 6 where it plateaued. The ADFI for diets T2, T3, T4 and T5 reached their maximum at week 7 where they remained stable. The least squares means for ADFI (g DM/kg $W^{.75}$ per day) of females was 120.5 ± 0.6 and for males 118.4 ± 0.6.

6.4.2 Growth performance

Body weights were influenced ($P < 0.05$) by week, treatment * week and treatment * sex interactions. Figure 6.2 shows the least squares means for sheep on the different diets. The body weights increased linearly, but with fluctuations by diet. Pairwise contrasts of body weights for sheep on different diets across the weeks were not significant (P > 0.05). Contrasts of the body weight at week 1 against the average body weights from weeks 2 to 13, indicated that peak body weight had not been attained (Littell *et al*., 1998) for sheep on diets T1, T2; while T4 showed a trend (P = 0.081). Sheep on diet T3, however, attained peak body weight at week 12 (P = 0.944); similarly those on diet T5 attained peak body weight at week 12 (P = 0.402).

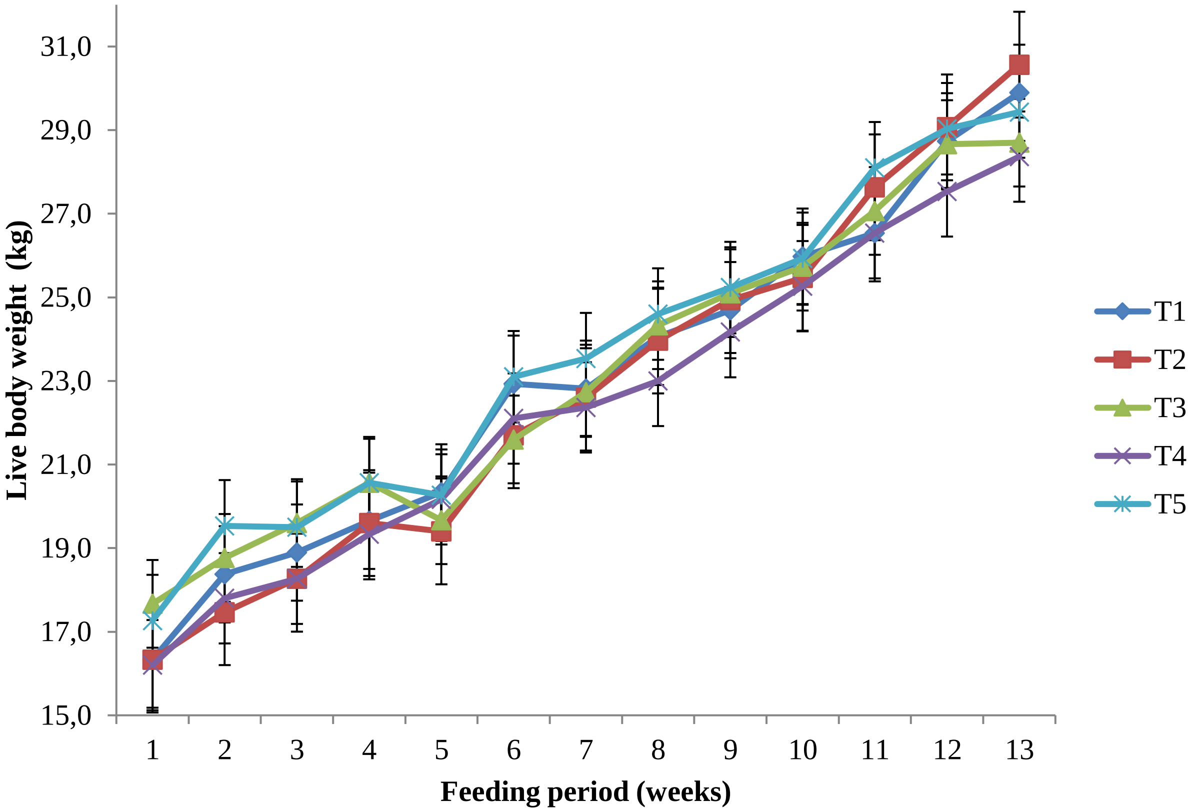

Figure 6.2 Average body weight (kg) of Damara lambs during the feeding period

* T1 = conventional feedlot diet; T2 = *Senegalia mellifera*-based diet; T3 = *Dichrostachys cinerea*-based diet; T4 = *Terminalia sericea*-based diet and T5 = *Rhigozum trichotomum*-based diet

Least squares means for treatment by sex interactions are shown in Table 6.2. With the exception of diet T4, there were no differences (P > 0.05) in body weights between males and females within in each treatment diet. Across diets, however, there were some differences in body weights. Male lambs on the control diet (T1) were heavier (P = 0.022) than males on the T4 diet. Females on T2 were heavier (P = 0.045) than males on T4 diet. Similarly, males on T2 were heavier (P = 0.039) than males on T4. Females on diet T3 showed a trend of being heavier (P = 0.05) than males on T4 diet. Males on diet T3 were heavier (P = 0.017) than males on T4. Females on diet T4 were heavier (P = 0.0001) than males. Females on T5 were heavier (P = 0.011) than males on T4. Males on T5 were heavier (P = 0.020) than males on T4.

The average daily gain (ADG) and feed conversion ratio (FCR) were affected (P < 0.05) by sex and treatment. The least squares means of ADG and FCR of the lambs fed the five different diets are presented in Table 6.2. Treatments T2 gave higher (P < 0.0001) ADG than other treatments. The least squares means for ADG (g/day) were 124.9 ± 4.3 for females and 153.5 ± 4.3 for males. T2 had a lower (P < 0.05) FCR compared to other bush-based treatment groups (T3, T4 and T5), but a trend (P = 0.098) of a better FCR was observed when T2 was compared to the control group T1. The FCR for T3, T4 and T5 were also similar (P > 0.05) to the control group T1. The FCR for females was 9.8 ± 0.3 and for males was 8.1 ± 0.3.

Table 6.2 Mean (± SEM) of growth variables for Damara lambs fed five diets from different roughage sources

	Treatment diets*						
Variable	T1	T2	T3	T4	T5	SEM	P-value
Initial weight (kg)	16.2	16.3	17.7	16.2	17.3	0.774	0.5546
Final weight in (kg)	29.7	30.6	28.7	28.4	29.4	0.870	0.4317
Total gain (kg)	13.5	14.2	11.8	12.2	12.2	0.832	0.2363
#Average daily gain (g/day)	148.0^{b}	156.4^{a}	124.2^{b}	133.7^{b}	133.7^{b}	6.9	< 0.0001
#Feed conversion ratio	8.8^{ab}	7.6^{b}	9.5^{ab}	9.6^{a}	9.4^{a}	0.5	< 0.0001
#Body weights (kg) for males	23.4^{a}	22.9^{a}	23.5^{a}	19.8^{b}	23.3^{a}	1.0	0.005
#Body weights (kg) for females	22.7^{b}	22.8^{b}	22.7^{b}	25.0^{a}	23.8^{b}	1.0	0.005

* T1 = control diet; T2 = *Senegalia mellifera*-based diet; T3 = *Dichrostachys cinerea*-based diet; T4 = *Terminalia sericea*-based diet; and T5 = *Rhigozum trichotomum*-based diet; $^{a-d}$Means with different superscripts within a row differ significantly (P < 0.05); # Least squares means; SEM = Standard error of means

6.5 Discussion

6.5.1 Chemical composition of treatment diets

It is a general practice to include a minimum amount of roughage in high-concentrate feedlot diets to maintain rumen health and reduce digestive disorders (Mertens, 2002). According to Jolly and Wallace (2007), ruminants appear to differ in their minimum fibre requirements; sheep in particular require a minimum of 10% roughage in the diet. However, in this study, all diets contained 40% roughage from different sources. Mertens (2002) cautioned against feeding an excessive amount of fibre because it can increase rumen fill and reduce the dry matter intake (DMI), which subsequently also reduces animal growth.

The five treatment diets were also formulated to provide the similar amount of protein (13% CP) and energy (9 MJ/kg ME), as per the nutritional requirements of growing lambs proposed by NRC (2007) along with the corresponding body weights and expected growth rates of lambs. There was some slight deviation from the targeted CP and ME values, but this was inevitable given the differences in batch composition of the grass bales and milled bushes due to different proportions of small branches and leaves.

In this study, the NDF contents of the diets were 50% or below and was lower than the concentration suggested (60 - 65%) to limit intake and digestibility of nutrients in ruminants (Van Soest *et al*., 1991b). Van de Vyver *et al*. (2014) study also used feedlot diets for lambs with the NDF contents ranging from 24.3 – 42.1%, where Lucerne hay was replaced with different inclusion levels of maize silage as a roughage source. The NDF contents of the five treatment diets used in this study (Table 6.1) were, however, higher than the recommended range of 15 to 20% NDF by Smith (2008).

6.5.2 Feed intake

Feed intake is a major factor that influences the amount of nutrients available to the lamb in order to realise its growth potential (van der Merwe *et al.*, 2020). A key concern of high proportions of roughage in feedlot rations is the high NDF content, which may physically restrict the dry matter intake through rumen fill (Jolly and Wallace, 2007). The lambs on the T2 and T3 treatment diets initially struggled to increase their voluntary feed intake possibly due to the physical nature of the fibre and the high NDF content as reported in Table 6.1, compared to the other diets. Even though the lambs increased their intake as they grew, they were still not able to reach the same DM intakes over the 90-day study period as the sheep on the T4, T1 and T5. Intake is a function not only of the NDF concentration, but also of the source of fibre (Ruiz *et al*., 1995), which partly explains the variability in intake of diets formulated from different roughage sources.

It was noted that the sheep on diet T2 and T3 showed a high tendency of selecting and sorting their diets during feeding. In both the morning and afternoon feeding sessions, they first selected and ingested the concentrate mix, and then sorted small particles of the roughage materials. This observation was in agreement with Jolly and Wallace (2007) who stated that feed sorting was a common practice in feeding management in feedlots. It was suggested that this can reduce the prediction of the physically effective NDF (peNDF) adequacy of the fed diet. The physically effective NDF (peNDF) of a feed is defined as the fraction of neutral detergent fibre (NDF) that stimulates rumination and contributes to a proper ruminal digesta mat consistency (Mertens, 2002). Even though peNDF was not specifically considered in this study, its principles may be useful in understanding the observed sorting and selective behaviour on feed intake for lambs fed different treatment rations. Kröger *et al*. (2019) also reported a similar observation on feed sorting in a commercial dairy herd.

On the other hand, the T4 and T5 treatment groups were observed to consistently consume their feed at a higher rate than the T2 and T3 among the bush-based treatment groups with minimal or no selection, indicating that their physical fibre structure could be blending in well with the concentrates mix, which discouraged or limited sorting behaviour. It could also possibly indicate a positive effect on the palatability of the diet. However, the palatability aspect may still need to be quantified. Treatment diets T4 and T5 had lower NDFom, which possibly contributed to a faster rate of digestion, hence reducing rumen fill. Treatment diet T4 may also have been consumed in higher proportion relative to T2 on account of the higher NDF digestibility reported in Table 4.1 (Chapter 4), resulting in faster clearance from the rumen. Other factors including high palatability and high passage rate (Jolly and Wallace, 2017) may have contributed to their greater intake than T2 and T3.

Although lambs in the control group diet recorded the highest feed intake throughout the 90- day feeding period, the observed high feed intake for the first five weeks, was exaggerated by the accompanying high wastage. The grass quality used in the preparation of the total mixed ration for T1 appeared to have been too light and less dense than milled bush used for the bush-based diets as a roughage source, which led to the bulkiness of diet T1. Due to this bulkiness, lambs fed on the T1 diet kept burrowing under the feeding trough searching for concentrate mix while feeding, therefore the consistent sorting and wasting behaviour of lambs, resulted in the lower feed refusal and higher calculated feed intake. This is because not all feed particles could be recovered as refusal as some may already be mixed with urine and faecal waste by the time of collection

Due to observations made about the feeding behaviour and wastage, interventions were made in week 6 by using the same quantity of water (200 ml) for all diets, to moisten and bind the feed ingredients, which minimized sorting and selection of feed components, hence resulting in better estimates of dry matter intake. Indeed the contrast of treatment mean of the first week versus the average of the rest of the weeks show that the peak consumption occurs at weeks 6 - 7 for all diets (Littell *et al*., 1998), which indicates the intervention was effective. Nonetheless, differences in gradual adjustment to the diets by the rumen microbes in the first weeks of the trials, cannot entirely be ruled out, despite the possible confounding effects due to the sorting behaviour of the sheep during feeding.

Overall, it was observed that the fibre characteristics (physical and chemical) of the different roughage sources used in the five treatment diets could have contributed to the acceptability of the diets by lambs and hence the observed variation in feed intakes of the different groups. However, in a study by Flores-mar *et al.* (2018), replacing alfalfa hay with sorghum straws as roughage source yielded similar dry matter intake, when diets were formulated to contain the same percentage of forage NDF. This was not the case in the current study, as the diets were only formulated to contain similar inclusion levels of roughage sources but not the NDF content. Differences in feed efficiency as indicated by residual feed intake (RFI) were explained by digestive differences among animals, but this also interacted with the type of diet offered (De La Torre *et al*., 2019). Although diet digestibility was not assessed in this study, it is plausible that intake may have been impacted by differences in diet digestibility attributable to varying ADLom content and rate of digestion.

According to the NRC (2007), the daily dry matter intake of fast growing lambs can vary between 3.8 and 4.2 % of live weight, which was confirmed by Jolly and Wallace (2007) for Australian lamb feedlots and van der Merwe *et al*. (2020) for South African lamb feedlots. The feed intake of lambs in this study was 1.5 kg DM /day, which is 5.1 % of live weight and may indicate greater feed wastage for sheep on bush-based diets, due to sorting.

6.5.3 **Growth performance**

The non-significant difference in the body weights of the weaned sheep on the control diet versus the rest of the diets across the 13 weeks, is surprising given that bush-based diets on account of their high lignin content as indicated by ADLom (Table 6.1), should have restricted intake and hence body weight gains. Other pair-wise comparisons in body weights across the trial period were also not significant showing apparently no differences among the diets. Using, however, another type of contrast shows there were treatment x week and treatment x sex interactions affecting body weights. The results indicate that peak body weight was reached at week 13 in diets T3, T4 and T5. The results suggest that these three bush-based diets as formulated, while able to meet maintenance requirements for weaned sheep, may constrain growth resulting in late attainment of mature size.

The treatment x sex interactions for body weight (kg) were most pronounced in diet T4 (25.0 ± 1.0 for females versus 19.8 ± 1.0 for males). The significant treatment xsex interactions for body weights implies there may be a differential response on the different diets possibly attributable to feeding behaviour differences in male and female sheep. The average daily gain was greater in males than in females and this is an agreement with other studies (Rodríguez *et al*. 2008). Unlike the body weights which showed treatment x week and treatment x sex interactions, ADG and FCR are summary measures that provide aggregate performance and may mask the inherent fluctuations and subtle differences among animals under different treatments. The differences in growth rates of males and females lambs observed in this study among the different treatment diets were similar to the observations by Rodríguez *et al*. (2008) in a fattening study on Assaf lambs, which could partly be associated with the dietary selection by animals of different sex. These authors found that the growth rates were lower in females than in males which also tended to be reflected by the similar trend in their dry matter and crude protein intake.

According to van der Merwe *et al.* (2020), for profitable production, producers often aim for an ADG of 300 g/day and FCR of 5.0 kg feed/kg weight gain, depending on the breeds and type of feed used in the finishing system. In this study, the ADG was much lower (with the best value being on T2 diet at 156.4 ± 6.9 g/day), while the best FCR was 7.9 ± 0.5 (on diet T2) which was far more inefficient than the proposed figures above. The basis for the lower ADG in the present study could be related to the high inclusion rate of low quality roughage sources than most conventional feedlot diets which usually contain 20% or less roughage. Other studies such as that of Van de Vyver et al. (2014) where Lucerne hay was replaced with maize silage as roughage source, reported similar FCR to T1 and T2 in this study. As indicated above, however, this level of FCR is not optimal for the production of sheep in a feedlot. Therefore, future research is warranted to optimize these diets to specification levels for growing lambs in the feedlot, with special focus on inclusion levels of each encroacher species depending on their physical and chemical characteristics of fibre to maximise their use. There may also be a need to chemically or enzymatically treat bush-based feed so as to improve nutrient utilization (Adesogan *et al.*, 2019).

6.6 **Conclusions**

Partial replacement of grass and Lucerne hay with milled bush of the different encroacher species in the diets fed to weaned lambs did not negatively affect their daily weight gain. Lambs fed T3 diet had overall higher ADG and better FCR than other treatment group. The feed intake for the treatment groups, however, varied significantly based on roughage sources included in the diets and the time point at feeding, which consequently increased the FCR. The physical properties of different roughage sources seems to have influenced the sorting and selecting behaviour as well as the DMI of Damara lambs. Therefore, more research is needed to develop efficient feeding strategies or processing such as pelletizing, that encourage inclusion of milled bush with different physical forms as roughage source. Furthermore, ADG and FCR of sheep could also be improved by formulating the diets at higher specified nutrient levels to sustain high growth rates. Although bush-based diets have the potential to serve as survival diets for weaned sheep, their use for production functions like growth may be restricted by lower digestibility.

CHAPTER 7

Slaughter weights and carcass characteristics of Damara lambs fed bush-based rations from four Namibian encroacher bush species

7.1 Abstract

The effects of feeding bush-based diets on slaughter weights and carcass characteristics of weaned Damara lambs were assessed in this study. Thirty Damara lambs with an average initial weight of 16.7 ± 1.9 kg, were used in a completely randomized experimental design with five treatments and six animals per treatment over a 90-day feeding period. A conventional diet was used as a control (T1), consisting of coarsely ground Lucerne (10%) and grass hay (30%) as roughage, while the other four treatment diets (T2-T5) consisted each of the bush species: *Senegalia mellifera*, *Dichrostachys cinerea*, *Terminalia sericea* and *Rhigozum trichotomum* at the same roughage inclusion rate (40%). All diets were formulated to contain the same crude protein (13%) and metabolizable energy (9 MJ/kg) contents. At the end of the feeding trial, the lambs were slaughtered and carcass characteristics were evaluated. The slaughter, hot carcass and cold carcass weights were influenced ($P < 0.05$) by sex, with males being heavier (30.7 ± 0.4, 12.0 ± 0.2 and 11.6 ± 0.2 kg) than females (28.0 ± 0.4, 11.4 ± 0.2 and 11.0 ± 0.2 kg), respectively. The subcutaneous fat, ultimate pH (pH 24) and *Longissimus dorsi* width were not affected ($P > 0.05$) by the treatment diets. Lambs fed T4 had greater ($P < 0.05$) *Longissimus dorsi* depth (2.2 ± 0.1 mm) and area (8.3 ± 0.5 mm^2) than the control group (1.7 ± 0.1 mm and 5.9 ± 0.5 mm), respectively. The dressing percentage ranged from 37.4 ± 0.8 to 40.0 ± 0.8 and it tended to be higher ($P = 0.087$) in female (39.0 ± 0.5) than male (37.8 ± 0.5) sheep. Therefore, the results of the study indicated that milled four encroacher bushes used could serve as alternative source of roughage to partially replace conventional roughage sources like grass and Lucerne hay resulting in similar dressing percentage and carcass characteristics of Damara lambs.

7.2 **Introduction**

The total population of small ruminants in Namibia is approximately 2 million (57%) sheep (Dorper, Damara and Swakara) and 1.6 million (43%) goats (Kandiwa *et al*. 2020). Sheep production in Namibia is concentrated in the more arid southern regions, especially Hardap and Karas regions, which account for 68% of the country's sheep population (Namibia Statistics Agency, 2015). Among the sheep breeds, the Damara is one of the important breeds especially in Southern Africa due to their resilience and adaptability to arid conditions (Almeida, 2011; Almeida *et al*., 2014). Damara sheep are fat-tailed and much of the body fat is accumulated around the tail with minor fat deposits in the rest of the body (Burger *et. al.*, 2013). This characteristic does not only give this breed its ability to survive in harsh conditions, but also potentially allows it to produce lean meat (Tsabalala *et al.*, 2003).

In Namibia, sheep are predominantly raised on natural rangelands. However, the availability and the nutritive values of the rangeland grasses decline in the dry seasons, resulting in seasonal body weight loss among the animals (Oosthuizen and Laubscher, 2019). The situation becomes even more critical during the drought years when there is little or no available standing forage. As a consequence, animals take longer to reach slaughter weights and often produce lower quality carcasses (Ben Salem *et al*., 2004). To cope with these seasonal changes, supplementary feeding has to be implemented, which can be expensive especially for small scale farmers. Given the existing challenges of low and highly variable rainfall, with periodic droughts in the country, finishing off slaughter sheep from natural pasture may not always be practical. This may imply that farmers may periodically or permanently resort to feedlot systems either on farm or at specialised enterprises, not only to maximize production but also to fatten and improve carcass quality of the animals before they are slaughtered.

In order to sustain commercial lamb and mutton production, rounding off of lambs in feedlot systems has been an increasing trend in countries such as South Africa (Brand *et al*., 2018; Van Der Merwe *et al*., 2020a). Kahuika *et al*. (2006) reported that South Africa has been the main export destination for Namibian sheep as live animals. However, since Namibia introduced the Small Stock Marketing Scheme (SSMS) in 2006, the Meat Board of Namibia (2011) reported that live exports have been declining and therefore new initiatives on value addition to local mutton production were implemented (Anon, 2011).

Carcass characteristics such as dressing percentage, carcass grade and yield are important when finishing lambs in a feedlot (Van der Merwe *et al*., 2020b). Apart from that, they further indicated that the diets of feedlot lambs had been found to be one of the main factors influencing the carcass characteristics and meat quality.

The feedlot diets usually include good quality roughage such as Lucerne hay (Van Der Merwe *et al*., 2020a). The shortage and high cost of Lucerne hay and other feedlot diet ingredients in the market, however, often limit their usage (Shivambu *et al*., 2011). Hence, finding affordable and sustainable alternative roughage sources that are locally accessible, would enable animals to produce good quality meat. This is not only to have enough in reserve in case of drought, but also to maximize production to supply sufficient quantities of slaughter sheep to abattoirs, which also meet the required quality and market standards.

In Namibia, fresh or dried milled bush from encroacher bushes has recently been recognised by farmers as roughage to sustain their livestock especially during drought and presents a potential valuable feed resource. In addition, Honsbein *et al*. (2017) evaluated the feeding value of *S. mellifera* milled bush as a replacement for grass hay in total mixed feedlot diets of cattle up to 500 g/kg of ration without negative effect on their growth, health and carcass characteristics. This clearly demonstrated that milled bush can replace conventional roughage sources such as Lucerne and grass hay in diets of fattening animals. However, the study by Honsbein *et al*. (2017) was only restricted to one bush species and hence there is need to explore the feeding value of other encroacher bush species in the diets of sheep.

There is limited scientific information on the productive performance especially carcass characteristics of sheep fed milled bush as roughage source under feedlot conditions. Therefore, the objective of this study was to evaluate the effect of feeding weaned Damara sheep with diets containing milled bush as roughage source from four different encroacher bush species namely: *S. mellifera, D. cinerea, T. sericea* and *R. trichotomum* on slaughter weights and carcass characteristics.

7.3 Materials and Methods

At the end of the growth trial (intake and growth performance study in Chapter 6), lambs were slaughtered. Therefore, the materials and methods prior to slaughter were similar to those described under Sections 6.3.1 - 6.3.7.

7.3.1 Study area

The experiment was conducted as per the study area described in Section 6.3.1.

7.3.1 Feed ingredients and preparation of experimental diets

This is described under Section 5.3.2 and 5.3.3 of Chapter 5, as per the ingredients composition of the experimental diets presented in Table 5.1.

7.3.1 Sampling and chemical composition analyses of experimental diets

The experimental diets fed to the lambs were analysed for chemical compositions as per the procedures described under Section 6.3.7.

7.3.2 Experimental animals and management

The experimental animals and their management has been described under Section 6.3.2.

7.3.3 Experimental design

The experiment was conducted using the experimental design described under Section 6.3.4.

7.3.4 Slaughtering and measurement of carcass characteristics

At the end of the feeding experiment, all sheep were individually weighed to obtain the final live weight (FLW), after being fasted overnight, with only access to water, to avoid digesta contamination. They were then transported to the Neudamm abattoir for slaughter, which is approximately 11 km from the experimental site.

7.3.4.1 Slaughter procedure

Sheep as described under Section 6.3.2 were slaughtered in accordance with the prescribed animal ethics and welfare regulations, at the registered abattoir facilities of the Neudamm campus, University of Namibia, under the required conditions for local abattoirs in Namibia. Animals were stunned to render them unconscious and to ensure that they did not suffer pain during slaughter. After stunning, sheep were slaughtered by cutting the carotids, jugular vein, oesophagus and trachea to allow blood to drain from the carcass. The carcasses were hanged on the bleeding rail and the dressing operation began after the bleeding process was completed. All slaughtering procedures were done under the supervision of a veterinarian, specialized in Veterinary Public Health.

7.3.4.2 Carcass and organs inspection

Overall inspection of carcases was done in accordance with guidelines of the Meat Safety Act of the Republic of South Africa (Act No. 40 of 2000) (Republic of South Africa, 2000) as adopted by Namibian abattoirs. After slaughtering, the head of each sheep was cut off, and then teeth and gums were inspected for any abnormal wear or corrosive signs that could be as a result of consuming the different treatment diets. Upon opening the carcases, all internal organs such as the heart, lungs, liver, spleen, kidneys (red offals) and the gastrointestinal tract (white offals) were removed and inspected for any abnormalities (Figure 10.5 and Figure 10.6 under Appendix).

The rumen, reticulum, omasum and abomasum were opened, emptied, and rinsed with cold water. Due to the rumen fermentation function, the focus was on the mucosa of the rumen. The examination and assessment of the rumen mucosal surface (Figure 10.6 under Appendix) were carried out as described by Suárez *et al*. (2006) and it was performed by a Veterinarian specializing in Veterinary Public Health and experienced in meat inspection. Briefly, the mucosal surface was visually examined and qualitatively assessed according to the presence and density of rumen papillae as follows: 1 = poor (small number of papillae or short papillae) and 2 = good (numerous papillae or long papillae). The presence or absence of plaque formation (rumen mucosa containing focal or multifocal patches with coalescing and adhering papillae covered by a sticky mass of feed, hair and cell debris), as referred to by Suárez *et al*. (2006) was visually assessed. The veterinarian had no prior knowledge of the treatments.

7.3.4.3 Carcass grading

After dressing and evisceration procedures, carcasses grading was done visually according to the South African Meat Classification System for beef, lamb, mutton and chevon, according to the Government Notice No. R.863 of 1 September 2006 (Republic of South Africa, 2006), which is also adopted by the Meat Board of Namibia. This system is used for grading animals at Namibian abattoirs. Quality grades as done by the grader combines three separate factors that affect the palatability and tenderness of the meat namely: maturity, subcutaneous fat and muscle conformation.

The carcass grading system uses three maturity groups, denoted by letters – A (represents the carcass from the youngest animal with no permanent teeth) to C (represents the carcass from older animal with more than six permanent teeth).

Fat was measured by means of visual appraisal into six fat classes on an increasing scale varying from score 0 (no visible subcutaneous fat on the carcass) to score 6 (excessively overfat – obese – carcass surface covered with subcutaneous fat).

The conformation was visually assessed on the muscular and skeletal formation of the carcass. Thus the conformation descriptions in the grade specifications referred to the thickness of muscling and the fullness of the carcass (Strydom and Smith, 2005). The conformation codes range between 1 (very flat) to 5 (very round).

7.3.4.4 Measurements of carcass characteristics

After dressing and evisceration procedures were completed, carcasses were weighed to obtain hot carcass weights (HCW). The pH of the carcasses was measured 1 hour (pH_1) after slaughter in the left *Musculus longissimus dorsi* at the area between 10th to 13th rib, using a portable digital pH meter with a penetration electrode and temperature probe (Figure 10.7 under Appendix).

Subsequently, carcasses were chilled in a cooler under the temperature of 2-4 °C for 24 hours to obtain the cold carcass weights (CCW). The pH reading of the carcasses was taken again at 24 hours (pH_{24}) in the *Musculus longissimus dorsi* on the same spot where pH1 was taken.

After 24 hours of chilling, the carcasses were then split into two halves down the spinal column by a longitudinal cut on the vertebral column using a meat band saw. The left side of the carcass was used for further measurements such as external length (CEL) of each carcass, shoulder and buttock circumferences (SC and BC, respectively), which were measured with a flexible tape measure following the procedure described by De Boer *et al*. (1974). A 3-rib cut (9th through to 11th ribs) in Figure 10.8 under Appendix was also removed from left side of each carcass for further dissection.

Fat thickness was measured at ¼ (Fat Pos¼), ½ (Fat Pos½) and ¾ (Fat Pos¾) positions on the 12th rib from the chine bone end, with a vernier calliper (Figure 10.9 under

Appendix) and from these, average fat thickness was calculated (Lima *et al.*, 2015; Araújo *et al*., 2017). The depth (eye-rib_D) and the width (eye-rib_W) of the *Musculus longissimus dorsi* also known as rib-eye muscle, exposed by cutting the carcass between the 12th and 13th ribs were measured with a Vernier calliper (Ferreira *et al.*, 2012; Landim *et al*., 2015), as shown in Figure 10.10 under Appendix.

As per the method described by Ferreira *et al*. (2012), the area of *Musculus longissimus dorsi* was determined by measuring the maximal width of the *longissimus dorsi* muscle, denoted as "A" and its maximal depth "B" as demonstrated in Figure 10.10 under Appendix and then applying the values into the following equation: $(A/2 \times B/2) \times \pi$, where π equals to 3.1416.

The three-rib cut (Figure 10.7 under Appendix) was weighed and physically separated by blunt dissection into bone, lean and fat tissue (Figure 10.11 under Appendix) which were weighed individually and expressed as a proportion of the cut as described by Van der Merwe *et al*. (2020). The proportion of each tissue was used as an estimation of the carcass composition.

Cold carcass weight (CCW) was expressed as a proportion of the live weight at slaughter (SW) to determine the dressing percentage (Van der Merwe *et al*., 2020).

7.3.5 **Statistical Analysis**

The data on carcass characteristics were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure (SAS, 2009). The main effects were treatment diets (T1-T5), sex and their interactions. Age at slaughter (days) was also considered in the model as a covariate. Data were checked for normality and transformation was carried out where necessary, before statistical analysis. The treatment x sex interaction effect was not significant for any of the traits considered in this study and thus was excluded from the final model.

The following statistical model was used:

$$Y_{ijk} = \mu + F_i + S_j + e_{ijk}$$

Where:

Y_{ijk} = dependent variable (HCW, DP, etc,)

μ = overall mean

F_i = effect of treatment diet (i = T1, T2, T3, T4 and T5)

S_j = effect of sex (j = male and female)

e_{ijk} = random error term explaining variation among experimental units (EU) on the same treatment

Means were separated using the Turkeys' Studentised range test. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences. In addition, differences among means with $0.05 \leq P \leq 0.10$ were accepted as representing tendencies to differences.

7.4 Results

7.4.1 Carcasses and organs inspection

Post-mortem inspection of carcases and organs showed no observed signs of abnormalities that could be as a result of possible ante-mortem infections. Therefore, there were no condemned carcasses or organs from any treatment groups in this study.

The results from the macroscopic evaluation of the rumen mucosa are shown in Figure 10.11 under Appendix. There were no incidences of plaque formation observed among the different dietary treatment groups. In addition, the visual assessment of the rumen mucosa from all five dietary treatment groups showed no difference in terms of well-developed papillae and healthiness of the mucosal wall and they were all given a score of 2. There were also no visible signs of physical damage or erosion of the rumen mucosa specifically on the rumen papillae (Figure 10.12 under Appendix) of the bush-based treatment groups (T2-T5) as compared to the control group (T1).

7.4.2 Carcass characteristics

7.4.2.1 Carcass grading

In the present study, grading of carcasses indicted that lambs from all five treatment groups visually scored similarly (score 1) in terms of subcutaneous fat cover, and they all fell under the same maturity group (A). Therefore, all the carcasses were awarded the A1 classification (Table 7.1) at the abattoir and thus, no statistical analysis was carried out since there was no variation amongst them.

7.4.2.2 Carcass measurements

The slaughter weight and carcass characteristics results are presented in Table 7.1. The diet treatment effects was not significant ($P = 0.086$) for hot carcass weight. The slaughter weight (SW), hot carcass weight (HCW), and cold carcass weight (CCW) were influenced ($P < 0.05$) by sex. Irrespective of the treatment diet, the SW, HCW and CCW were lighter ($P < 0.05$) for female lambs than male lambs, while the dressing percentage was not significant ($P = 0.087$) between female and male lambs.

None of the effects were significant ($P > 0.05$) for: carcass yield; average subcutaneous fat thickness and fat thickness taken at three different positions over the *Musculus* *longissimus dorsi* (the rib-eye muscle); pH_{24}; carcass lean; carcass external length (CEL); buttock circumference; and rib-eye width. The model fit was poor ($R^2 < 0.35$).

The rib-eye depth was influenced ($P = 0.045$) by treatment, with carcases of lambs on T4 had greater ($P = 0.003$) rib depth than those on T1. Carcases of lambs on T2 and T3 showed a tendency ($P < 0.080$) to have more rib depth than those on T1. Slaughter age showed a tendency ($P = 0.052$) to influence rib depth and the regression coefficient was 0.010 ± 0.005. Treatment diets affected ($P < 0.05$) rib-eye area (REA). Lambs on treatments T1 and T5 had lower ($P < 0.05$) rib eye area (REA) than those on T4. Slaughter age tended ($P = 0.09$) to influence REA and the regression coefficient was 0.042 ± 0.023.

The pH_1 values were influenced ($P < 0.05$) by treatment and slaughter age. Lambs on treatment T5 had higher ($P < 0.05$) pH_1 values than those on other treatments. The regression coefficient of pH_1 on slaughter age was 0.012 ± 0.004.

The diet treatment effect and sex did not ($P > 0.05$) have influence on the proportion of muscles : bones : fat (Table 7.2) among the treatment groups.

Table 7.1 Slaughter weight and carcass characteristics least squares means (±SEM) of the Damara lambs fed different diets

Variables	**Treatment (T)**					**Sex (S)**		**P values**		**R-Square (R^2)**
	T_1	T_2	T_3	T4	T5	F	M	Treatment	Sex	
Carcass grade	A1	A1	A1	A1	A1					
SW (kg)	29.7±0.9	30.6±0.7	28.7±0.7	28.4±0.7	29.4±0.7	28.0±0.4^b	30.7±0.4^a	0.174	0.001	0.52
HCW (kg)	12.3±0.3	12.3±0.3	11.3±0.3	11.3±0.3	11.5±0.3	11.4±0.2^b	12.1±0.2^a	0.086	0.030	0.38
CCW (kg)	11.9±0.3	11.8± 0.3	10.9± 0.3	10.9±0.3	11.0± 0.3	11.0± 0.2^b	11.6± 0.2^a	0.114	0.047	0.35
CCD (%)	40.0±0.8	38.4±0.8	37.7±0.8	38.4±0.8	37.4±0.8	39.0±0.5	37.8±0.5	0.178	0.087	0.30
CEL (cm)	56.7±0.8	54.8±0.8	54.8±0.8	54.0±0.8	54.2±0.8	54.7±0.5	55.1±0.5	0.184	0.651	0.23
SC (cm)	21.8±0.4	22.0±0.4	21.2±0.4	20.3±0.4	21.0±0.4	21.1±0.3	21.4±0.3	0.053	0.477	0.30
BC (cm)	34.8±0.6	35.2±0.6	35.0±0.6	34.0±0.6	35.2±0.6	34.8±0.4	34.9±0.4	0.665	0.906	0.09
pH$_1$	5.6±0.1^b	5.9±0.1^b	5.8±0.1^b	5.8±0.1^b	6.2±0.1^a	5.8±0.1	5.9±0.1	0.012	0.737	0.44
pH$_{24}$	5.3±0.04	5.3±0.04	5.3±0.04	5.3±0.04	5.3±0.04	5.3±0.03	5.3±0.03	0.860	0.280	0.10
Fat_1(mm)	3.3±0.5	4.1±0.5	2.6±0.5	2.7±0.5	2.9±0.5	3.5±0.3	2.7±0.3	0.313	0.120	0.28
Fat_2 (mm)	2.7±0.4	2.5±0.4	2.0±0.4	2.4±0.4	1.8±0.4	2.3±0.3	2.2±0.3	0.303	0.591	0.19
Fat_3 (mm)	2.8±0.4	2.0±0.4	2.2±0.4	3.1±0.4	2.4±0.4	2.7±0.3	2.3±0.3	0.275	0.299	0.22
Avg fat (mm)	3.0±0.3	2.8±0.3	2.3±0.3	2.8±0.3	2.4±03	2.9±0.2	2.4±0.2	0.434	0.131	0.20
Rib eye-width (cm)	4.4±0.1	4.5±0.1	4.7±0.1	4.7±0.1	4.5±0.1	4.5±0.1	4.6±0.1	0.289	0.505	0.19
Rib eye-depth (cm)	1.7±0.1^b	2.0±0.1^{ab}	2.0±0.1^{ab}	2.2±0.1^a	1.9±0.1^b	-	-	0.045	-	0.34
Rib eye area (cm^2)	5.9±0.5^b	7.1±0.5^{ab}	7.2±0.5^{ab}	8.3±0.5^a	6.8±0.5^b	-	-	0.038	-	0.35

* T1 - conventional feedlot diet; T2– *Senegalia mellifera*-based diet; T3 - *Dichrostachys cinerea*-based diet; T4- *Terminalia sericea*-based diet and T5 - *Rhigozum trichotomum*-based diet. SW = slaughter weigh; HCW = hot carcass weight; CCW = cold carcass weight; CCD = cold carcass dressing; CEL = carcass external length; SC = shoulder circumference; BC = buttock circumference; SEM = standard error of the mean; F = Female; M = Male. $^{a-b}$Means with different superscripts within a row differ **(**P < 0.05**).**

Table 7.2 Proportion of the muscle, bone and fat (LSM±SEM) of the 3-rib cut from carcasses of the Damara lambs fed different diets

Variables	***Treatment (T)**					**Sex (S)**		**P values**		**R-Square (R^2)**
	T1	T2	T3	T4	T5	F	M	Treatment	Sex	
Lean (%)	46.1±2.3	50.3±2.2	47.3±2.2	49.9±2.2	48.0±2.2	47.5±1.4	49.1±1.4	0.707	0.443	0.13
Fat (%)	23.8±1.9	20.8±1.9	20.3±1.9	23.0±1.9	21.3±1.9	23.0±0.2	20.7±1.2	0.659	0.176	0.15
Bones (%)	24.2±2.2	25.5±2.1	27.4±2.1	22.3±2.1	26.2±2.1	24.0±1.3-	25.3±1.3	0.367	0.840	0.21

* T1 - conventional feedlot diet; T2– *Senegalia mellifera*-based diet; T3 - *Dichrostachys cinerea*-based diet; T4- *Terminalia sericea*-based diet and T5 - *Rhigozum trichotomum*-based diet; LSM-least square mean; SEM-standard error of the mean

^{abcd}Means with different superscripts within a row differ significantly **(**P < 0.05**)**

7.5 Discussion

7.5.1 Carcasses and organs inspection

Post-mortem inspection of carcasses and organs at abattoirs form part of the hygienic processing of meat before it is passed and declared fit for human consumption (Alemayehu *et al*., 2013; Ishmael *et al*., 2017). This is mainly done to remove grossly visible conditions and contaminations from the food chain that may pose a risk to public health (Madzingira *et al*., 2018). Since there were no observed health risk conditions or contaminations on carcasses and organs from any treatment group in this study, all carcases were approved to be fit and safe for human consumption.

In addition to the general health inspection of the carcasses, this study also focused on a macroscopic inspection or evaluation of the gastrointestinal tract to test the hypothesis that bush-based diets may cause erosion and physical damage especially to the morphological structure of the rumen papillae. A shift in diet with different energy density and physical features influences morphology of ruminal epithelium (e.g. degree of papillation and volume capacity) and in its environment (e.g., microbial population) (Beharka *et al*., 1998; Steele *et al*., 2011; Liu *et al*., 2013; Steele *et al*., 2015; Wang *et al*., 2017; Ma *et al*., 2017; Mason *et al*., 2019).

Roughage from different encroacher species used in the preparation of bush-based diets, constituted a significant proportion of wood in addition to leaves. Hence, in comparison with conventional feed like grass hay, it was hypothesised that the physical and chemical fibre structures of milled bush will be rough in nature and high in NDF content, which may pose negative morphological changes to the rumen epithelium. However, findings from this study showed no evidence linking the bush-based diets (40% dried milled bush: 60% concentrate) to abrasion of the rumen epithelium morphology. This is in agreement with findings by Steele *et al*. (2011) and Liu *et al*. (2013) that fed high fibre diets (100% roughage: 0% grain or concentrate); or by feeding low quality forage such as rice straws and corn stovers (Wang *et al*., 2017); or large fibre particle size of >8 to 19 mm (Beharka *et al*., 1998; Ma *et al*., 2017; Wang *et al*., 2017).

Instead, the opposite has been observed with high grain and energy feeding in ruminants associated with detrimental ruminal environment, depicted by high short chain fatty acids concentration, low pH value, resulting in sub-acute ruminal acidosis and high papillae lesion scores in goats (Liu *et al*., 2013) and dairy cattle (Steele *et al*., 2011; Ma *et al*., 2017; Wang *et al*., 2017). The later studies further indicated that high grain fed animals showed profound alterations in ruminal epithelial tissues depicted by noticeable epithelial cellular damage and intercellular junction erosion, which compromised the structural integrity of the rumen epithelium. This may interfere with absorption of nutrients and efficient utilisation of feed (Steele *et al*., 2011; Liu *et al*., 2013; Wang *et al*., 2017). The results from this study also showed no evidence of rumen papillae abrasion or differences among the treatments due to different roughage sources, based on the macroscopic evaluation of the rumen papillae.

Other related research work on the effect of fiber or roughage on rumen development indicated that roughage promotes rumen muscle development and also maintains the integrity of the rumen epithelium in both young and mature ruminants (Booth, 2003; Suárez *et al*., 2007; Steele *et al*., 2011; Liu *et al*., 2013; Trulla, 2013; Ma *et al*., 2017). It has also been demonstrated that there is a large amount of variation in papillae development with respect to diet chemical composition and physical properties (Suárez *et al*. 2007; Steele *et al*. 2011; Liu *et al*. 2013; Ma *et al*., 2017).

In the present study, the macroscopic evaluation of the rumen clearly showed the beneficial effects of roughage, irrespective of source, to reduce incidence of plaque and of poorly developed mucosa (Figure 10.11). This is in agreement with observation by Suárez *et al*. (2007) on the effect of various roughage sources, concentrate to roughage ratios fed in addition to a milk replacer diet on rumen development and growth performance of veal calves. They concluded that that adequate physical stimulation (“roughage scratch factor”) is required to maintain a healthy rumen mucosa and to stimulate rumen motility and consequently the proper development of the mucosal wall. Nonetheless, both extremes (i.e. high concentrate diets and all roughage diets) have negative consequences on the rumen mucosal development and maintenance (Booth, 2003) and should therefore be avoided.

7.5.2 **Carcass characteristics**

7.5.2.1 Carcass classification

Carcass classification systems were developed to inform processors and consumers about the quality of the carcass and thus to distinguish its market value (Brand *et al*., 2018). In the present study, all carcasses of the Damara lambs in the five treatment diets fell in the same age category (A: with no permanent incisors) and fatness score 1, which gave an overall A1 grade. The age category was expected to be similar since they were all born within the same lambing season. The A1 grading could be explained by the fact that Damara sheep are fat-tailed and much of the body fat is accumulated around the tail with minor fat deposits in the rest of the body (Kleemann *et al.*, 2000; Tshabalala *et al*., 2003; Almeida, 2011; Wilkes *et al.*, 2012; Almeida *et al*., 2014). It was further argued that the fat-tail breed characteristic does not only give it the ability to survive in harsh condition by using stored fat to mobilize energy, but also suggests its potential to produce lean meat. Therefore this implies that the milled bush diets from the studies shrubs are good enough to produce lean meat.

When considering the subcutaneous fat results, it is important to note that Damara sheep breed is late maturing in terms of meat production and fat deposition potential (Almeida *et al*., 2014). Therefore, irrespective of the nutritional influence, it is expected that Damara would have leaner meat and could require an extended rearing period to achieve high and similar degrees of fatness, compared to an improved meat sheep breed like Dorper, which is known to be early maturing and deposits high levels of fat at an earlier age (Van der Merwe *et al*., 2020).

According to Louvandini *et al*. (2006) carcasses finished with good grades tend to fetch a better price/kg at sale, especially in countries with a tradition of lamb and mutton production, as it is the case for Namibia and South Africa. For example in the South African market, there is a high demand for lamb from young sheep (with no permanent incisors, Age Class A) and a lean fat cover (fat depth of 1–4 mm; Fat Class 2) (Van der Merwe *et al*., 2020) and so a premium price is offered for carcasses that meet these specifications. Hence, from the viewpoint of a lamb producer, it is important to meet the market demands in order to maximize income from production and sustain optimum mutton profitability. Van der Merwe *et al*. (2020) reported that South African lamb producers make use of feedlot finishing soon after weaning in order to add value to their lambs in preparation for slaughter to obtain a premium price for lamb carcasses.

7.5.2.2 Carcass measurements

Slaughter, hot carcass and cold carcass weights were heavier in males than females which is consistent with findings by Simela *et al*. (2011) in goats and Van der Merwe *et al*. (2020) in lambs. It should be pointed out that other than slaughter weight and pH_1, the model fit for all other variables was poor ($R^2 < 0.40$), implying that other unidentified sources of variation were more important in influencing the carcass variables than slaughter age, treatment, sex, and treatment x sex interactions.

Dressing percentage was in the range 37.4 to 40%, which is similar to what was reported in Menz sheep (Assefa *et al.*, 2008), but this falls far short of dressing percentage reported in other studies for example: 46.1 – 48.3% (Cardoso *et al*., 2021); 49.4 – 50.2% (Rezaei *et al*., 2013); 59.9% (Simela *et al*., 2004). Dressing percentage is affected by live weight, fatness, time off water and feed, sex and breed (Warmington and Kirton, 1990). Higher growth rates have been typically associated with higher dressing percentages (Seoni *et al*., 2018) and given that ADG in this study was in the range 124 to 156 g/day, the slower growth could have contributed to the lower dressing percentage. Wilkes *et al*. (2012) reported dressing percentage in Damara sheep of 53.2 ± 1.9%, which reflected greater fatness compared to Merino (dressing percentage = 41.5 ± 1.8%). Results of this study suggest that while bush-based diets may be able to meet maintenance requirements, the inclusion rate used in this study might be high for supporting optimal growth in feedlots. Further studies are needed to determine optimal inclusion levels of browse material in feed rations and determine whether chemical or biological treatment may improve their utilization.

The pH_1 for T5 was slightly higher compared to other treatments which may be attributed to low muscle glycogen reserve possibly due to low energy supply (Vestergaard *et al*., 2000). Although the diets were formulated to be iso-energetic, diet T5 had the lowest DM and OM digestibility (Table 5.3, Chapter 5) which could have contributed to low energy reserves. It was, however, observed that the ultimate pH_{24} was similar for the different diets. Dietary energy intake impacts muscle glycogen reserves (Daly *et al*., 2006; De Brito *et al*., 2016) and these in turn affect muscle pH values. This implies that the observed similar ultimate pH could be attributable to a different factor not muscle glycogen reserves as influenced by dietary energy levels. Muscle pH values affect colour, water-holding capacity and sensory attributes of lamb meat (Ferguson and Gerrard, 2014). In this study muscle glycogen reserves were not determined and neither was the eating quality assessed but these would be useful in future studies to determine effects of different bush-based diets on meat sensory attributes.

Rib eye area (REA) is associated with the amount of muscle in a carcass and is indicative of muscle development and yield of high value cuts (Williams, 2002). The results indicate greater muscle development for animals on diets T2, T3 and T4 compared to T1 and T5; similarly rib eye depth was greater in T4 than T1. Even after adjusting for differences in CCW, the advantage of T4 over T1 and T5 were still evident for both rib eye depth and REA. It is not clear why the T4 diet could have positively influenced muscling without impacting ADG.

7.6 **Conclusions**

The results of the study showed that milled bush from the four encroacher bushes used in the feedlot diets could be potential sources of roughage to partially replace conventional sources like grass and Lucerne hay. The lambs fed the bush-based diets had carcass characteristics that compared well with lamb fed with the control diet. Cold carcass weights, grading, ultimate pH, and fat thickness were similar among treatments. Lambs on the T4 diet produced carcasses with a greater rib eye area and depth. Although encroacher bush could be used to partially substitute grass hay, the low dressing percentage possibly related to slow growth rates is of particular concern because it impacts profitability. Further studies are needed to assess optimal inclusion levels of encroacher bush in feedlot diets and on the effect of encroacher bush inclusion on the meat sensory attributes.

CHAPTER 8

General discussion, conclusions and recommendations

8.1 General discussion

8.1.1 Chemical composition

Much of the substantial information now available on the chemical composition of bush species in literature is from leaves only (Abdulrazak *et al.*, 2000; Kamupingene *et al.*, 2004; Osuga *et al.*, 2008; Nassoro, 2014; Marius, 2016) and a few reports that were done on leaves and very soft twigs of ≤ 10 mm in diameter (Aganga and Mesho, 2008; Theart *et al.*, 2015). There is, however, limited information on the chemical composition of milled bushes, i.e. leaves and twigs of ≤ 20 mm in diameter which are widely used as bush feed by livestock farmers in Namibia. Species by season interaction was significant for chemical composition of encroacher bushes, which suggests differences in phenological stages (vegetative, flowering and seeding) at the time of sampling (Chapter 3). From a practical perspective, this suggests that timing of harvest of different encroacher bushes to process into ruminant feed may be crucial in optimizing nutrient utilization. Complementarity in nutrient composition could also be exploited by formulating diets from different encroacher bushes.

Species by season interactions affected DM, OM, ash, EE, CP, NDFom, ADFom, ADLom, hemicellulose, cellulose and the amino acid profiles. *Senegalia mellifera* and *D. cinerea* which are species from the same agroecological zone, showed generally similar trends in their chemical composition by season. Thus, their CP peaked during the early rainy season, NDFom and ADFom were lowest during the early rainy season, ADLom was highest during the late rainy season, hemicellulose was lowest during the early rainy season and cellulose decreased gradually from late dry season to late rainy season. Optimal nutrition composition for ruminant feeding for these two species would therefore be achieved by harvesting during the early rainy season.

Based on the chemical composition it would appear that *R. trichotomum* would be best harvested during the late dry to early rainy season to optimize its nutritive value for ruminant feeding. The chemical composition trends for *T. sericea* are less clear and more studies are needed. Notwithstanding the above recommendations, timing of harvesting is dependent on available biomass and would also be influenced by the rainfall pattern in a specific year.

The data on the chemical composition has indicated that all four bush species in the current study had low to moderate crude protein (CP) ranging from 46.9 g/kg DM in *T. sericea* to 111.8 g/kg DM in *S. mellifera* across all three seasons. In general, ruminant feeds with the crude protein contents below the minimum threshold of 6 - 7% like those reported for *T. sericea* in this study are considered deficient because they cannot provide the minimum ammonia concentration required for microbial activities and growth in the rumen (Kaitho, 1997). The implication is that while encroacher bushes may be able to meet maintenance requirements to support rumen microbial function for some seasons of the year, supplementation with other protein sources is required during the late dry season (May – November). The crude protein contents of species in this study were lower than those found in leaves only (10.4 - 20.3% CP) of the same species (Abdulrazak *et al*., 2000; Kamupingene *et al*., 2004; Osuga *et al*., 2008b; Nassoro, 2014; Marius, 2016). This was expected because of the dilution effect from the thicker twigs (15 -20 mm) included in the milled bush, which contain high fibre and less protein. However, the crude protein of roughage from encroacher bush species in this study compares well to some grasses from natural pasture available to animals in the dry season (2.1 - 5% CP) and in the wet season (4 - 11.8%) reported from some communal areas of Namibia by Schneiderat (2011).

The NDF and ADF concentration in this study varied from 594.3 to 734.3 g/kg DM and 463.2 to 578.6 g/kg DM respectively, with *R. trichotomum* having the lowest contents while *D. cinerea* had the highest. All fibre fractions i.e. NDF and ADF (Table 3.1) were higher for all species (> 500 g/kg DM) than for those of the same species reported by Kasale (2013), Theart *et al*. (2015) and Marius (2016), who mainly sampled leaves or leaves with small twigs of less than 15 mm stem diameter. The stem size harvested or included in bush samples of this study i.e. 20 mm ("broom stick size") was done to mimic the current practice by farmers of producing milled bush feed in Namibia as reported by Honsbein *et al*. (2017). Given the higher stem: leaf proportion of the milled bush samples used in this study, the exceptionally high fibre fractions observed was in line with expectations. Fibre is important from the ruminant nutritional point of view, but high concentration of fibre fractions in the diets has the greatest influence both on the digestibility and dry matter intake of the given diet (Jung *et al.*, 1997; Mertens, 2002; Smith, 2008).

In addition to dietary fibre content, other key physical factors affecting DM intake are fibre digestibility and the rate of fibre degradation in the rumen (Roche *et al.*, 2008). Dado and Allen (1996) suggested that increased NDF digestibility was positively correlated with voluntary feed intake. Research has shown that increasing ADF or NDF over 180 – 200 g/kg DM and 600 g /kg DM may limit feed intake due to rumen fill (Santini *et al.*, 1992; Mertens, 1994; Bakshi and Wadhwa, 2007; Riaz *et al.*, 2014). Therefore, high NDF levels (> 600 g/kg DM) observed in this study imply that incorporation of encroacher bush in ruminant diets should be limited because of its adverse effect on intake and digestibility. Therefore, both the amount and chemical composition of the fibre are important in determining the minimum fibre requirements for ruminant diets and the effect of fibre on production.

Also consistent with expectations, high lignin contents (138 - 223 g/kg DM) obtained in this study are generally more common in most tropical bush shrubs than in herbaceous plants (Njidda, 2011), but the content varies according to species, stage of growth, age and the plant parts. For example, Scogings *et al*. (2004) observed lower cellulose and lignin in other bush species during growth (2 - 12% and 0.5 - 9%) than during plants dormancy (3 - 17% and 0.5 - 14%) respectively. Lignin is one of the major phenolics that is bound to all plant cell walls, and is a significant limiting factor in the digestion of plant materials in the rumen (Njidda, 2011).

8.1.2 **Protein fractioning**

Protein fractioning results from this study (Table 3.2) indicated that bush species such as *S. mellifera* and *R. trichotomum* had high potential rumen degradable protein (NPN and STP) ranging from 51 - 57 g/100g CP while other species such as *D. cinerea* and *T. sericea* had a high fraction of potential rumen undegradable protein (B fractions) ranging from 64 - 72 g/100g CP. This could imply that depending on the proportions of different fractions, some bush species in this study might need protein supplements with more degradable protein than others in order to overcome the depressing effect of low nitrogen concentration in the rumen. This is a major finding that can be used in incorporating bush based feeds in fodder flow planning on Namibian rangelands.

Difference in solubility and degradability of the dietary crude protein among the bush species are primarily associated with varying levels of tannins (Table 3.3) and fibres (Table 3.1) and the extent to which they bind with protein and/or enzymes thus reducing their digestibility (Fall-Toure and Michalet-Doreau, 1995; Kaitho, 1997; Ngwa, 2002; Soul, 2017).

In addition, Kaitho (1997) further emphasized that crude protein content of the feed does not fully account for their nutritive value as protein sources without considering their nitrogen degradability in the rumen and intestinal digestibility of the undegradable protein. Hence, the new systems of the protein evaluation partition the dietary protein into rumen degradable and undegradable protein (Mahesh *et al*., 2017). The ammonia and amino acids produced when proteins get digested in the rumen are used for microbial protein synthesis, which contributes to the metabolizable protein requirements of ruminants. Similarly, the dietary protein that escapes the rumen undegraded also get digested in the intestines to provide the major source of absorbed amino acids for ruminants (Kaitho, 1997; Ngwa, 2002; Mahesh *et al.*, 2017; Soul, 2017).

8.1.3 **Anti-nutritional factors: total phenols and tannins**

The levels of CT obtained in all four bushes (Table 3.3) were relatively low (< 55 g CT/kg DM) and are reported to be the safe and upper beneficial limit in animal feeding and nutrition (Rubanza *et al*., 2005; Osuga *et al*., 2008). Hence negligible negative effects of CT could be expected from feeding milled bush of these species. Caution, however, needs to be exercised in use of *T. sericea* because of its high HT content in both the early and late rainy seasons.

The CT at lower concentration levels of 2 - 3% in ruminant diets are associated with reducing the degradation of useful protein in the rumen by the formation of protein-tannin complexes which later dissociate in the abomasum to supply the needed amino acids during protein digestion in the small intestines (McMahon *et al.*, 2000; Salem, 2005; Akande *et al.*, 2010; Makkar, 2010). In agreement with the observations in this study, it appears that varying levels of condensed tannins in bush species were associated with varying fractions of potential protein that are degradable and undegradable in the rumen. Thus, bush species such as *S. mellifera* and *R. trichotomum* with low levels of CT (0.9 - 3.2 g/kg DM) had more potential rumen degradable protein

(51 - 57 g/100g CP) and less undegradable protein (43 - 49 g/100g CP). On the other hand, other species such as *D. cinerea* and *T. sericea* with increased levels of CT (4.5 - 24.8 g/kg DM) had less potential rumen degradable protein (28 - 36 g/100g CP) and more of potential rumen undegradable protein (64 - 72 g/100g CP).

8.1.4 **Macro and micro minerals**

The concentration of most macro and micro minerals (Table 3.4) were considered sufficient for livestock maintenance except for P, Na, Cu and Zn (Nassoro, 2014)). Growing livestock require 0.28% Ca and the species had concentrations of at least 0.9% which was sufficient to meet their needs. The Mg content of *R. trichotomum* was below the 0.09% DM required for growing livestock. The low Mg concentration could imply a possibility for increased risk of induced hypomagnesaemia (Dove *et al.*, 2016; Masters *et al.*, 2018).

Feed deficient in P, Na, Cl and S have been associated (to varying extents) with a limitation in microbial growth (Ngwa, 2002) especially, P and S which are implicated in the metabolism of nucleic acids and amino acids, respectively, in microbes. All encroacher bush samples had low Na concentration which should be 0.06% DM. However, it is a common practice among livestock farmers in Namibia to supply animals with common salt (NaCI) block which can easily meet the Na deficiency. The K: (Na + Mg) ratio was 5.6 for *R. trichotomum* and more than 6 for *D. cinerea*, *S. mellifera* and *T. sericea*; hence the latter three species exceed the maximum recommended threshold of 6 (Newell *et al.*, 2020).

Mineral concentrations generally, increased from the late dry to early rainy season. The significant increase in the early rainy season could be associated with the moisture availability which facilitates absorption of nutrients. The implication of such variation is that while encroacher bushes may be able to meet mineral requirements for some seasons of the year, supplementation is required during the late dry season (May – November), when mineral concentrations are low.

8.1.5 **Amino and fatty acids**

Total amino acids and fatty acids for all species (Table 3.5 and Table 3.6) were relatively low but species presented an appreciable profile of essential fatty acids which are associated with good fatty acids in animal products for human health. The contents of total amino acids (AA) of species reported in this study ranged from 39.4

to 77.7 g/kg DM with *S. mellifera* showing the highest AA concentration in the rainy season, while *T. sericea* showed the lowest in both seasons. The inherent characteristics of each species differ in relations to its ability to extract and accumulate nutrients from soil and/or to fix atmospheric nitrogen, as in the case for leguminous plants (González-Andrés and Ceresuela, 1998; Scogings *et al*., 2004; Njidda, 2011). This was demonstrated by the high amino acids composition observed for *S. mellifera*, which is classified as a leguminous plant compared to other species.

In all species studied, the essential amino acids contributed about 42% of the total AA. This may imply that a greater proportion of essential AA of bush-based feed would need to be supplied through protein concentrates or supplements as undegraded dietary amino acids or from microbial amino acids synthesis in the rumen. The contribution of forages to AA supply is generally overlooked, especially in the diets of animals with high nutritional requirements such as dairy cows (Edmunds *et al*., 2013). However, knowledge of the AA profile in forage is still essential for accurate diet formulation and precision feeding (Edmunds *et al*., 2013; Tedeschi *et al*., 2001). Therefore, the results of this study also serve to increase the knowledge of AA profiles of milled encroacher tropical bush as roughage sources.

Overall, the variability observed in the nutrient content of four species presented in Chapter 3, could be attributed to within species variability associated with factors such as plant age, plant part, harvesting method, season and location (Larbi *et al*., 1997; González-Andrés and Cineresuela, 1998). Irrespective of the species and season which are the main effects considered, the results of the chemical composition in this study has indicated that milled bush is generally of low nutritional value and can hardly meet the maintenance requirements of the animals as a sole feed.

8.1.6 ***In vitro* organic matter digestibility and *in situ* neutral detergent fibre**

The milled bush from the different encroacher bush species differed widely in the digestible fractions of their respective OM with values ranging from 294.0 to 424.2 g/kg DM and NDF with values ranging from 118.6 to 264.3 g/kg NDF (Chapter 4). Digestibility of forage is greatly influenced by the fibre fractions (NDF, ADF and lignin), which in turn relates to the proportion and extent of lignification of plant cell wall (Kaitho, 1997; Larbi *et al*., 1997; Smith, 2008; Soul, 2017). Kaitho (1997), Smith (2008) and Soul, (2017) have reported that the lignification of the cell walls is a major factor restricting the digestion of fibre. In line with that, low digestible OM and NDF observed in this study can be attributed to the high fibre fractions especially lignin reported in Chapter 3. However, this was expected because of the higher stem: leaf proportion included in bush samples, which should contain high cell wall fractions compared to leaves alone.

In vitro OM digestibility (g/kg OM) was in the range 294.7 ± 3.2 to 424.4 ± 3.2 which compares poorly to tropical grass species which ranged from 420 to 510 g/kg (Mayhuddin, 2008) and 406 to 604 g/kg (Gemeda and Hassen, 2014). All encroacher bush species had relatively low *in vitro* OMD and neutral detergent fibre digestibility which constrains them as a sole feed resource for ruminants and may require chemical or enzyme treatments to improve their utility (Adesogan *et al*., 2019). For instance Ndozi (2020) reported that chemical treatment of *S. mellifera* with 8% urea reduced ($P < 0.05$) the ADF concentration.

Except for *S. mellifera*, the *in vitro* OM and *in situ* NDF digestibility estimates of other species significantly decreased from late dry to early rainy season. This observation was not expected because generally rainy season stimulates new flushes which possibly dilute the high fibre fractions in the stem (Scogings *et al*., 2004). Hence, an increase in digestibility as in the case of *S. mellifera* was expected for the other species. The possible reason could be insufficient soil moisture as a result of less or delayed rainfall in some study areas (Figure 3.2 and 3.3), despite the fact that it was a designated rainfall season. Overall, the high variability in the OM and NDF digestibility observed could be attributed to species differences and within species factors such as age, plant part harvested, season of harvest and locations (Larbi *et al*., 1997; González-Andrés and Cineresuela, 1998; Njidda, 2011).

8.1.7 **Methane production of encroacher bush species**

Methane production from the four encroacher bush species was evaluated in two different seasons (Chapter 4). *In vitro* methane gas production of all four species was higher during the late dry season compared to the early rainy season (147.6 versus 92.0 mL/g DM). This further supports our recommendation of harvesting during the early rainy season, to optimize nutrient composition for ruminant feeding and reduce methane emission from enteric fermentation. The enteric production of greenhouse gases (GHG) particularly methane by livestock and its impact on the environment is a global concern because methane contributes to global warming (Theart *et al.*, 2015; Macome *et al*., 2017). In addition, methane production by ruminants is associated with significant loses of energy in the rumen during the utilization process of feed energy (Theart *et al*., 2015). Therefore, reducing enteric methane from ruminants has become a focus of animal nutrition, especially in countries where agriculture is a major economic enterprise.

Methane gas production in this study is consistent with the reported higher methane production for highly fibrous feeds compared to concentrates (Adesogan *et al*., 2019). However, the methane gas values were higher than those reported by Theart *et al*. (2015), in some Kalahari browse species which ranged between 1.58 and 64 mL/g DM and by Gemeda and Hassen (2015) in some tropical browse plants which ranged from 0.10 to 20.67 mL/g DM. Goel and Makkar (2012) indicated that there is evidence that methane (CH_4) production is increased when feeds high in fibre are fed to ruminants. This is consistent with the high methane production observed in this study, taking into consideration the high fibre fractions of the browse species reported in Chapter 3.

The *in vitro* methane gas production (mL/g) of digested DM at 24 hours, decreased from 132.4 ± 6.6 for the late dry season to 92.7 ± 6.6 for the early rainy. It has been shown that the use of plants which contain secondary compounds such as tannins have anti-methanogenic properties (Kaitho, 1997; Theart *et al*., 2015; Macome *et al.*, 2017; Soul, 2017) and tended to reduce ruminal methanogenesis. The increased HT concentration from the late dry season to the early rainy season across all species reported in Chapter 3 may have contributed to the reduction in methane gas production. However, it has been suggested by Macome *et al*. (2017) that the anti-methanogenic effects also depends on the dietary concentration of secondary compounds. It has been reported that inhibition of methanogenesis by tannins is mainly due to the suppressed fiber degradation which could be due to a reduction in the number of cellulolytic bacteria or formation of tannin-cellulose complexes (Macome *et al*., 2017).

8.1.8 **Milled bush from encroacher bush species as roughage sources**

Milled bush materials from encroacher bush species was evaluated as roughage sources in diets of Damara lambs (Chapter 5). As indicated in Table 5.1 of Chapter 5, the ingredients composition of the diets used in this study showed that they had different roughage sources but at similar levels (40% DM basis). The physical form of the fibre (effective fibre) has been addressed in the present study by grinding all roughage sources to similar particle sizes of 10 mm commonly applied for lamb finishing diets (Smith, 2008).

Roughage sources have different physical and chemical characteristics with associative effects that influence the intake and digestibility of other ingredients in a mixed diet (Galyean and Hubbert, 2014). The NDF method used in Chapter 3 to measure fibre, only measures the chemical characteristics of fibre for ruminants. It does not, however, measure the physical properties such as particle size, which also influence the effectiveness of fibre in meeting ruminant minimum fibre requirements (Mertens, 2002). Roughage plays a vital role in ruminant diets for maintaining animal productivity and health. In addition to being an important energy source, it stimulates chewing and salivation that contribute to maintaining the proper rumen pH and health, and thus helps to avoid metabolic disorders such as acidosis (Mertens, 2002).

8.1.9 **Nutrients intake, apparent digestibility and nitrogen balance**

DM intake has been identified as the most important factor in ruminant animal production, determining the amounts of nutrients available for production, hence also the gross feed conversion efficiency (Roche *et al.*, 2008). The dry matter intake of the *S. mellifera* –based diet (T2) was similar to that of the *D. cinerea*-based diet (T3) despite the former having a lower NDFom, ADFom and ADLom. In addition, the lower ADFom digestibility of T3 compared to T2 (0.368 ± 0.027 vs. 0.510 ± 0.027 respectively, Table 5.3) , should also have resulted in greater rumen fill for animals on T3, but the intakes of *D. cinerea* and *S. mellifera* diets did not differ.

Galyean and Hubbert (2014) reviewed that much of the effect of roughage source and level on DMI in studies done with feedlot cattle could be accounted for by changes in the dietary NDF supplied by the roughage. Much of the observed variation in the nutrient intake and digestibility in the current study (Table 5.2) could therefore, be attributed to the concentration of NDF in the roughages as reported in Table 3.1. For example, the depressed intake of DM was greater on bush-based diets with NDF level (> 550 g/kg DM), than for the control diet (T1) which was a grass-based diet. Nonetheless, the higher digestibility of NDFom and ADFom for diets based on *S. mellifera* (T2) compared to others may have contributed to better nutrient utilization as reflected in the faster rate of gain for sheep fed on T2.

The lower apparent digestibility for DM, OM and CP for T4 and T5 diets (Table 5.3) could be attributed to the higher concentration of total phenolics (Table 3.3) in *T. sericea* and the high concentration of indigestible neutral detergent fibre (Table 4.1) in *R. trichotomum* respectively. Similar observations of reduced digestibility associated with high tannin contents were reported by Dschaak *et al*. (2011); Kardel *et al*., (2013) and Dickhoefer *et al*., (2016). In addition, T4 and T5 had higher ADLom contents, NDFom and ADFom intake than other bush-based diets (T2 and T3), in Table 5.3 which could have contributed to the lower digestibility observed. In agreement with this observation, Traxler *et al*. (1998), Moore and Jung (2001) and Soul (2017) also reported low digestibility in forage with high ADL content.

All treatments in this study had positive nitrogen balance which ranged from 16.2 to 22.1 g/day, which translate into 45.5 to 58.2% of the N intake. Even though the N intake and the N lost through feaces was similar among the treatments, sheep fed T5 diet retained significantly lower N, compared to the other four diets. The possible explanation to lower N retention of T5 could be linked to the significantly high N lost through urine. This indicates the rapid ruminal digestion, resulting in ammonia production in excess of microbial needs. Mui *et al*. (2002) reported that ammonia in excess of recycling needs is absorbed into the bloodstream, converted to urea in the liver and excreted in the urine. Protein fractioning results from this study (Table 3.2) also indicated that the *R. trichotomum*-based diet (T5) had high potential rumen degradable protein (NPN and STP) at 51 g/100g CP, which could explain the possible excess ammonia production in the rumen.

8.1.10 **Feed intake and growth performance**

Although all treatment diets had similar inclusion level of roughage, the ADFI for T1 exceeded ($P < 0.05$) that for T2 and T3, at most time points (weeks). The significant variation in feed intake by lambs during the feeding period in this study may be attributed to the variation in the chemical composition, especially the fibre fractions (NDF, ADF and ADL) content of diets (Table 6.2). The high NDF content (>400 g/kg DM) in the total mixed rations is a key concern, because it physically restricts the dry matter intake through rumen fill (Jolly and Wallace, 2007). In addition, the lower NDF digestibility (0.54 to 0.59) observed for the bush-based diets (T2-T5) might also indicate that the structural carbohydrates (NDF) from bush-based roughage sources might not be as digestible compared to NDF from T1. This could have ultimately increased the mean retention time (MRT) and contributed to the lower DMI of the lambs. Similar to the findings in this study, Smith (2008) reported low DM intakes associated with low NDF digestibility of *Eragrostis curvula* hay compared to *Medicago sativa*.

Roughage sources have different physical and chemical characteristics with associative effects that influence their intake and for other ingredients in a mixed diet (Galyean and Hubbert, 2014). Thus, feed intake is not only a function of the NDF concentration, but also of the physical nature of the roughage source (Ruiz *et al*., 1995), which may partly explain the variability in intake of diets observed with the different roughage sources used in this study. It is apparent that the observed lower feed intake of some bush-based diets was as a result of the selecting behaviour against the roughage particles. Physical factors likely contributed to suppression of feed intake especially for T2 and T3 as the sheep could not reach the same DM intakes over the 90-day study period as those on the T4, T1 and T5 diets. The sheep on diet T2 and T3 showed a high tendency of selecting and sorting their diets during feeding, indicating that their physical fibre structure could not blend in well with the concentrates mix as compared to others, which encouraged the sorting behaviour (Mertens, 2002; Jolly and Wallace, 2007; Smith, 2008; Whitney and Lupton, 2010; Salinas-Chavira *et al*., 2013; Galyean and Hubbert, 2014).

Results may also suggest that feed intake was restricted by the high NDF level (> 550 g/kg DM), which restricts available nutrients for growth. This could have further contributed to the observed low average daily gain (ADG) of 124.2 to 156.4 ± 6.9 g/day and poor feed conversion ratio (FCR) of 7.6 to 9.6 ± 0.5 kg feed/kg weight gain achieved in this study. Despite the positive response to different roughage sources, the performance was far more inefficient than the proposed figures by van der Merwe *et al*. (2020) for profitable production, where producers often aim for ADG of 300 g/day and FCR of 5.0 kg feed/kg weight gain. However, the performance will also depend on the breeds and type of feed or rations used in the finishing system. The roughage sources in this study were included at 40% in rations, which is far higher than the 20% or less roughage inclusion in conventional feedlot diets. Research is therefore warranted on optimal inclusion levels of each encroacher species depending on their physical and chemical characteristics of fibre to optimize growth.

8.1.11 **Carcass characteristics**

The overall low dressing percentage (37.4 to 40%) obtained in this study, is possibly related to slow growth rates and is of particular concern because it impacts profitability. Warmington and Kirton (1990) reported that dressing percentage impacts on the financial value of a carcass and is influenced by many factors including age, weight, plane of nutrition, degree of gut fill at slaughter, head and skin weight, fatness and dressing procedures (Mahgoub and Kadim, 2004; Simela *et al*., 2004, 2011).

In agreement with that, Speijers *et al*. (2009) reported that lamb carcasses from sheep fed on a higher plane of nutrition had higher dressing percentage compared to those on a low plane of nutrition, which was attributed to higher levels of subcutaneous fat. In addition to dressing percentage, many factors also affect meat quality including breed, age, sex, weight and diet (Webb *et al*., 2005). One important meat quality aspect is muscle pH, which influences the shear force, water retention capacity, cooking weight loss, flavour and colour of meat (Webb *et al*., 2005; Costa *et al*., 2012; Cardoso *et al*., 2021). It is recommended that the pH_1 of goat meat be lower than 6.4 and pH24 has to be in the range 5.4 – 5.7 for meat intended for commercialization (Maltin *et al*., 1998; Hamdi *et al*., 2016). Corazzin *et al*. (2019) suggested that ultimate pH > 5.8 indicated dark and tough meat with a shorter shelf life. The ultimate pH of the meat in this study was 5.3 ± 0.04 which suggests the bush-based feeds did not adversely affect meat quality.

In the present study, all carcasses of the Damara lambs in the five treatment diets fetched the same carcass grade (A1) which signifies that all lambs were within the same age category (A: with no permanent incisors) and had the same subcutaneous fatness score (1: a very lean carcass). Lean carcasses in Damara sheep breed are expected because they are fat-tailed and much of the body fat is accumulated around the tail with minor fat deposits in the rest of the body (Kleemann *et al.*, 2000; Tshabalala *et al*., 2003; Almeida, 2011; Wilkes *et al*., 2012; Almeida *et al*., 2014).

In countries with a tradition of lamb and mutton production, as it is the case for Namibia and South Africa, there is a high demand for lamb from young sheep (with no permanent incisors, Age Class A) and a lean fat cover (fat depth of 1 - 4 mm; Fat Class 2) (Van der Merwe *et al*., 2020). Corazzin *et al*. (2019) reported that meat from young and lean carcasses have characteristics that can meet the expectations of a consumer who is ever more aware of the health and nutraceutical aspects of food. On the other hand, from the commercial viewpoint, a premium price is offered for carcasses that meet these carcass specifications, particularly for A1 and A2 grades, thus maximizing income and increasing profitability from lamb and mutton production (Louvandini et al., 2006; Van Der Merwe et al., 2020a).

In summary, even with those identified physical and chemical challenges of different fibre sources, replacing conventional roughage (grass and Lucerne hay) with milled bush in diets of lambs at the level of 40% (DM basis) supported acceptable level of feed intake, performance and carcass characteristics. Overall, the *S. mellifera* diet may have been advantageous over the other bush-based diets due to the higher *in vitro* OM digestibility; better essential amino acid (Histidine and Lysine) profile; and higher ADFom digestibility contributing to the faster ADG (Table 6.3). This may imply that those roughages with higher NDF concentrations should be included at lower levels in diets; further studies are needed to assess optimal inclusion levels of encroacher bush in feedlot diets.

8.2 **General conclusions**

In conclusion, the results from this study have indicated that milled bush from encroacher bush species had moderate CP except for *T. sericea* which was below 50 g/kg DM. However, the encroacher bush species had high contents of fibre and lignin across all seasons, which is associated with low digestibility and thus has limited potential as a sole source of feed. Except for Ca, all bush species in the current study had low concentrations of macro minerals below the minimum requirements for ruminants, which may necessitate mineral supplementation of bush-based diets. The concentration of some micro minerals were sufficient to meet the minimum ruminant requirements of (Fe and Mn), but Cu and Zn were below the recommended levels for ruminant diets. The concentration of total amino acids and fatty acids were in the ranges of 39.4 to 77.7 g/kg DM and 1.17 to 2.84 g/kg DM, respectively, with *S. mellifera* having the highest concentration, while *T. sericea* showed the lowest.

A moderate proportion of soluble protein presented by *R. trichotomum* and *S. mellifera* could promote good ruminal functioning, while other species (*D. cinerea* and *T. sericea*) had a high proportion (>70 g/100g CP) of their CP bound to fibre and have a greater probability of being part of the slowly degradable protein or escaping the rumen as rumen undegradable protein. The concentrations of anti-nutritional factors were within the levels regarded to be safe for animal production and health. The low levels of the polyphenols may not require addition of tannin binding additives such as PEG in bush based feeds.

Despite some nutritional shortcomings in milled bush from the studied species, they have potential to be used as roughage source and can fully replace traditional roughage sources such as grass and Lucerne hay in properly formulated ruminant diets especially under drought conditions for survival purposes. Bush-based diets at the inclusion rate of 40% milled bush as roughage source, can support ADG of up to 156.4 ± 6.9 g/day for growing lambs and yield carcasses of 11.4 - 12 kg with exceptional quality standards of A1 grade, according to the Namibian carcass grading standards.

Species by season interactions affected DM, OM, ash, EE, CP, NDFom, ADFom, ADLom, hemicellulose, cellulose and the amino acid profiles. From a practical perspective, the timing of harvest of different encroacher bushes to process into ruminant feed may be crucial in optimizing nutrient utilization. Based on the chemical composition, *S. mellifera* and *D. cinerea* which were harvested from the same agroecological zone, showed generally similar trends in their chemical composition by season: CP peaked during the early rainy season. Optimal nutrition composition for ruminant feeding for these two species would therefore be achieved by harvesting during the early rainy season. *Rhigozum trichotomum* would be best harvested during the late dry to early rainy season to optimize its nutritive value for ruminant feeding. The chemical composition trends for *T. sericea* are less clear and more studies are needed. Notwithstanding the above, timing of harvesting is dependent on available biomass and would also be influenced by the rainfall pattern in a specific year.

Although encroacher bush could be used to partially substitute grass hay, the low dressing percentage possibly related to slow growth rates is of particular concern because it impacts profitability; further studies are needed to assess optimal inclusion levels of encroacher bush in feedlot diets and also effect of encroacher bush inclusion on the meat sensory attributes.

8.3 **Recommendations**

Following the current results, it is recommended that:

1. In order to optimize the quality of roughage from encroacher bush species to consider harvesting season, as the level of nutrients can be influenced by the species by season interactions. The results from this study suggest that harvesting should be done during the early rainy to late rainy season because of higher nutrient levels compared to the late dry season.
2. The CP content for encroacher bushes varied by season, implying that while encroacher bushes may be able to meet maintenance requirements to support rumen microbial function for some seasons of the year, supplementation is required during the late dry season (May – November).
3. It is not advisable to feed roughage from encroacher bush species as sole feed because it will not be able to meet the maintenance requirements of the animals due to the observed low CP contents, high fibre contents (NDF, ADF and ADL), low OM and DM digestibility. Improvement in DM and OM digestibility and nutrient contents can be achieved by adding concentrate supplements to the roughage. However, the inclusion rate of roughage from

encroacher bush species in the final ration should be guided by the desired level of animal production, but in any case not exceeding 40%. Hence further research should consider a lower inclusion level, less than 40%, of the milled bush in the ration in order to have improved animal live weight gains.

4. Although encroacher bushes are a promising roughage source, their high fibre content is of particular concern. Therefore, interventions that directly degrade fibre to release the energy and other nutrients contained in the milled bush should be considered for future research. For example, future research to investigate the effect of chemical treatment, duration of treatment and chemical concentration levels on fibre digestibility is required.
5. Complementarity in nutrient composition could also be exploited by formulating diets from different encroacher bushes.
6. Partial replacement of milled bush with other roughage sources such as grass or brewers grain requires investigation.
7. Although the effects of encroacher bush species as roughage sources seem relatively clear from this study, little is known about their effects at different roughage levels and physical form in bush-based diets; thus, additional research is needed to more fully address the role of these factors.
8. In addition, more research is also needed to quantify the effects of total NDF concentration in relation to the source of NDF in bush-based diets, on the nutrient intake, digestibility, growth, carcass characteristics and meat quality.
9. Although results of the encroacher bush species under investigation showed a low concentration of tannins, the utilization of protein and other nutrients in the bush-based feed is not yet fully established. Therefore, the degree to which the different concentration of tannins can be either beneficial or deleterious to the animals need to be investigated.
10. Future research should also consider the feeding of bush-based feed to other livestock species such as goats and cattle and determine the effects on growth and carcass parameters.

8.4 Implications and anticipated impact from the study

The work done in this study was aimed at providing evidence on the nutritional and feeding value of four encroacher bush species as an alternative feed source for livestock. The study also sought to address issues of forage shortage and related high costs which farmers frequently face due to recurrent droughts as experienced during the drought in 2019. The milled bush from encroacher bushes may not have sufficient nutritional value as sole feed to meet animal maintenance, but it can be effectively used as a moderate quality roughage source that can replace conventional roughage sources in the diets of livestock in years of drought. Milled bush can fully replace conventional roughages at 40% of the sheep rations on dry matter basis and support moderate growth rates and yield carcass characteristics of exceptional standard.

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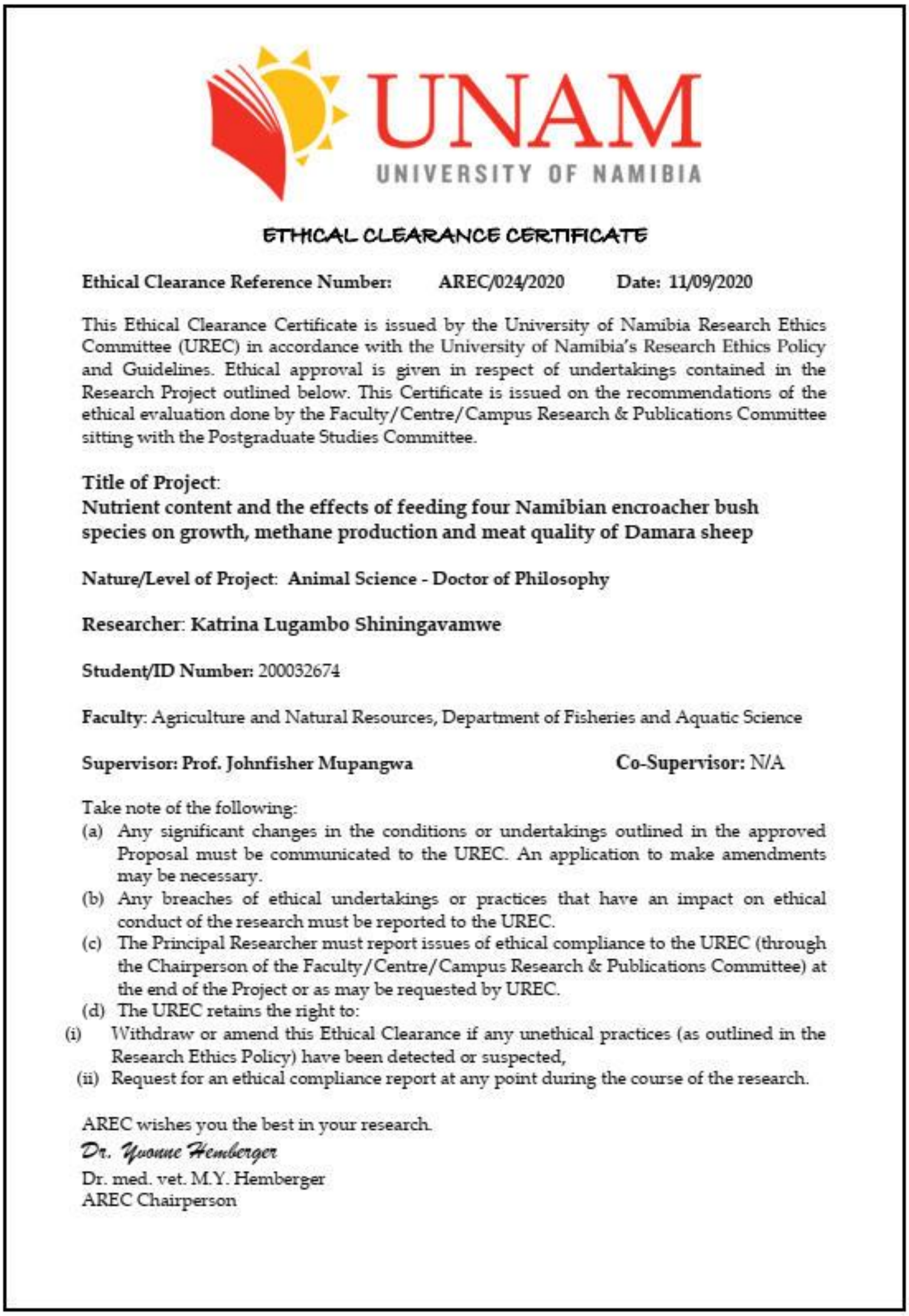
Appendices

10.1 Appendix 1 Ethical Clearance Certificate



UNAM
UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: **AREC/024/2020** **Date: 11/09/2020**

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project:
Nutrient content and the effects of feeding four Namibian encroacher bush species on growth, methane production and meat quality of Damara sheep

Nature/Level of Project: **Animal Science - Doctor of Philosophy**

Researcher: **Katrina Lugambo Shiningavamwe**

Student/ID Number: 200032674

Faculty: Agriculture and Natural Resources, Department of Fisheries and Aquatic Science

Supervisor: Prof. Johnfisher Mupangwa **Co-Supervisor:** N/A

Take note of the following:

(a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.
(b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.
(c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.
(d) The UREC retains the right to:
(i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
(ii) Request for an ethical compliance report at any point during the course of the research.

AREC wishes you the best in your research.

Dr. Yvonne Hemberger
Dr. med. vet. M.Y. Hemberger
AREC Chairperson

Figure 10.1 Ethical clearance certificate

10.2 Appendix 2 I*n vitro* and *in sacco* methods of feed evaluation

Figure 10.2 The process of the *in vitro* OMD, adding cellulose buffer solution to samples

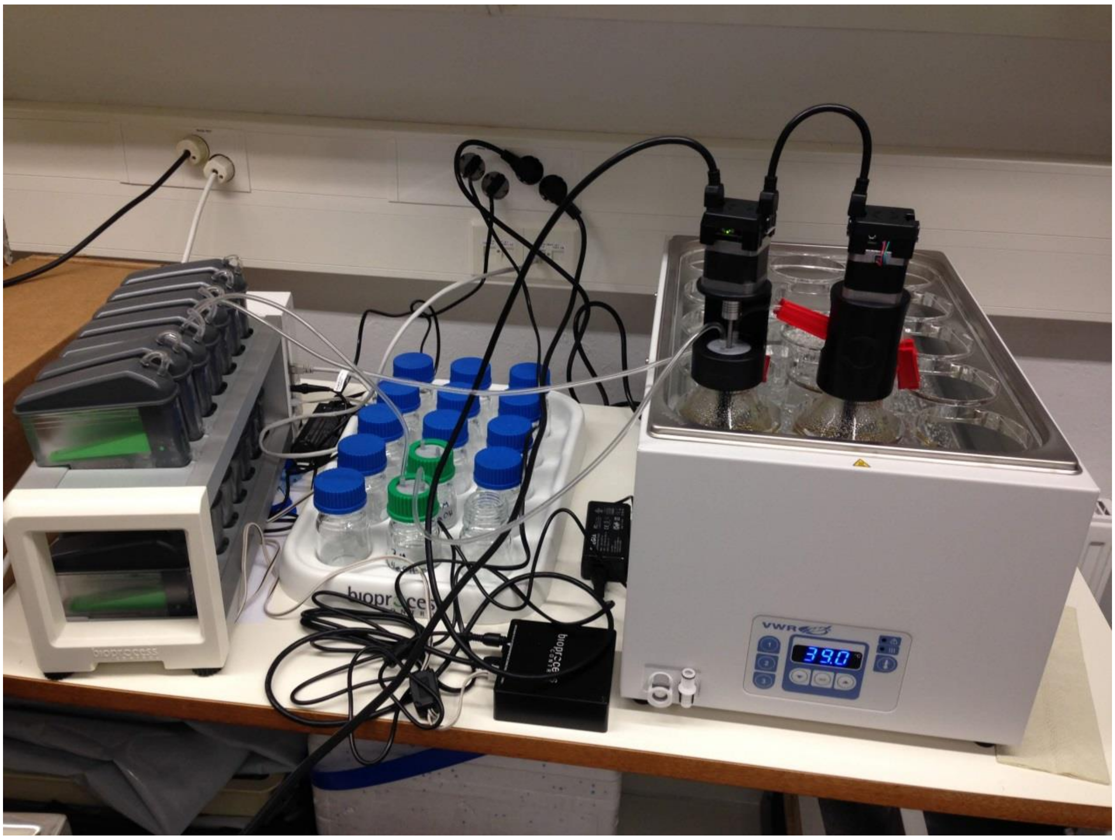


Figure 10.3 Gas Endeavour Automatic Gas Flow Measuring System

Figure 10.4 Prepared nylon bags with samples (left) before being inserted in the rumen of a fistulated cow (right)

10.3 Appendix 3 Organs inspection and carcass evaluation

Figure 10.5 Inspection of the red offal from the slaughtered lambs

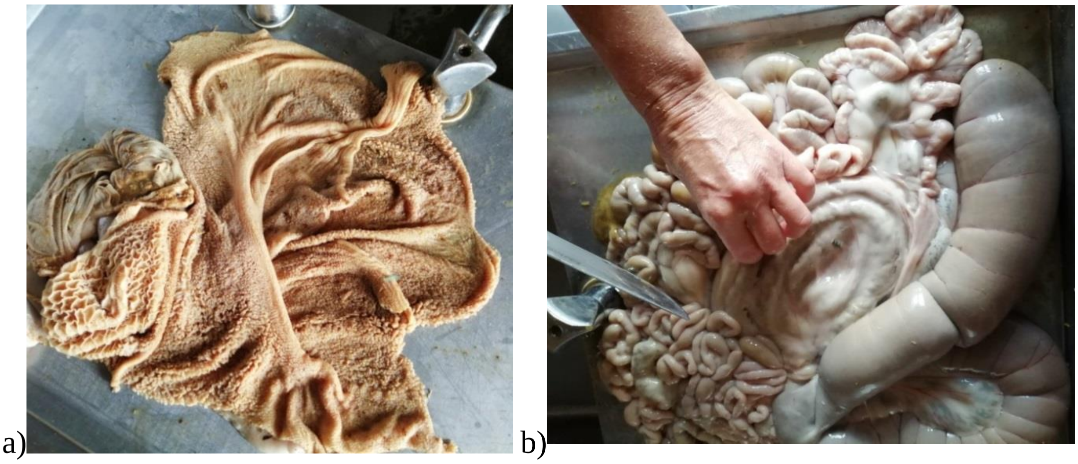


Figure 10.6 Inspection of the gastrointestinal tract mainly the stomachs (a) and intestines (b)

Figure 10.7 pH of the carcass measured in the *Musculus longissimus dorsi*

Figure 10.8 A 3-rib cut removed from the left side of the carcass for one of the lambs

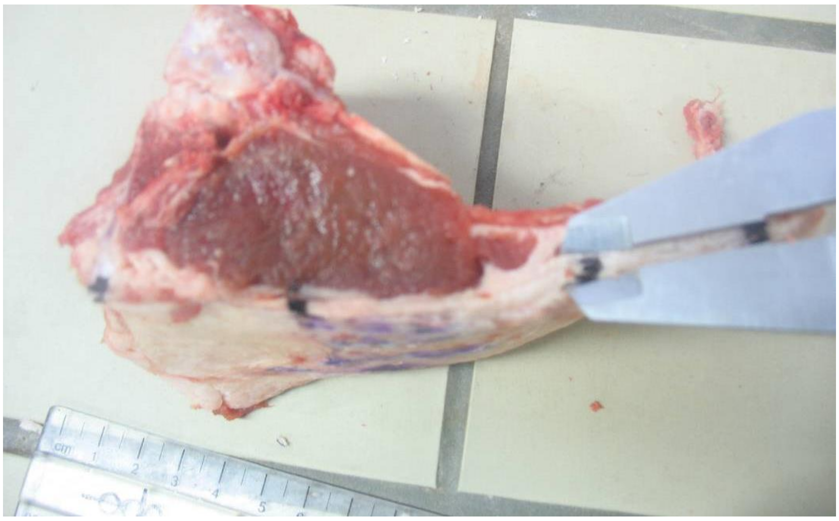


Figure 10.9 The marked positions on the 12th rib where the fat thickness was measured

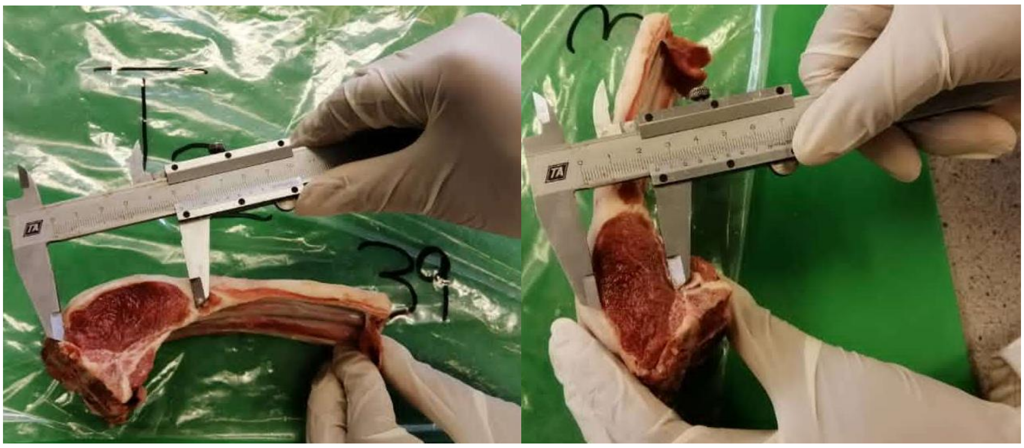


Figure 10.10 The width and depth of the *musculus longissimus dorsi* measured with a calliper

Figure 10.11 The muscle, fat and bone tissues dissected from 3-rib cut.

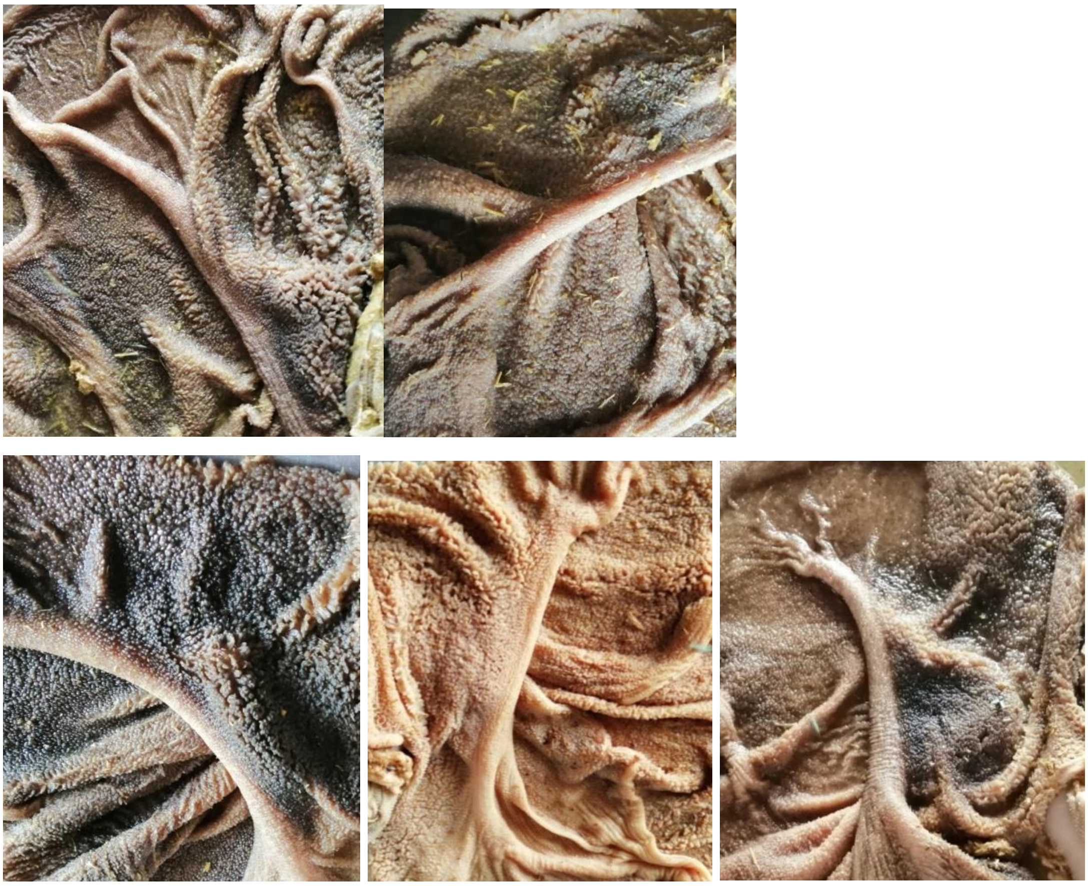


Figure 10.12 Rumen section from different treatment groups showing the papillae, from left to right (T1-T5), respectively