EFFECTS OF CHEMICAL TREATMENT ON NUTRIENT CONTENT AND PALATABILITY OF *SENEGALIA MELLIFERA* AND *CATOPHRACTES ALEXANDRII* BUSH-BASED FEEDS

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ABSTRACT

The objectives of this study were to determine: (1) the chemical composition of untreated *Senegalia mellifera* and *Catophractes alexandrii*; (2) the effect of NaOH and urea treatment at different levels (0%, 4%, 6% and 8%) on chemical composition of *S. mellifera* milled samples; (3) the nutrient composition of formulated diets from chemically and fibrolytic enzyme treated *S. mellifera* samples; (4) palatability of the feeds developed from *S. mellifera*, when fed to weaned Damara sheep. Samples consisting of small branches and twigs were analysed for chemical composition. Samples of *S. mellifera* were also treated for 21 days and the chemical composition was determined. Feed formulated from treated and untreated *S. mellifera* was assessed for palatability over 12 days in Damara sheep. The results showed that *S. mellifera* had a higher ($P < 0.05$) concentration of CP, NDF, ash and Ca than *C. alexandrii*. The concentration (g/kg DM) for *S. mellifera* were: CP (147.42 ± 0.37), NDF (653.63 ± 0.41) and ash (52.27 ± 0.11). Chemical composition (DM, OM, CP, EE, ADF, NDF, ash) of treated *S. mellifera* was influenced ($P < 0.0001$) by treatment. Treatment with 4% NaOH, 4% urea, 6% urea and 8% urea increased ($P < 0.05$) CP concentration compared to control. Formulated diets differed ($P < 0.05$) in their DM, OM, EE, NDF, ADF and ash. The control diet had higher ($P < 0.05$) NDF concentration compared to diets with 4% urea or 4% NaOH. Feed intake was influenced ($P < 0.05$) by day and diet x day interactions. Urea (8%) and NaOH (4%) treatment improved the chemical composition and palatability for *S. mellifera* feed, but the reaction conditions including chemicals, concentration, moisture content, pH and duration need to be optimized, to allow greater reduction of the lignin concentration.

Keywords: *Senegalia mellifera*, *Catophractes alexandrii*, encroacher bush, palatability, fibre.

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DEDICATION

This thesis is dedicated to my mother Maria Grace Ndozi and to my elder sister Shando Aldronicah Luswenyo, for their remarkable support, love, indispensable encouragement, imaginativeness and fruitful guidance during my childhood up to now. I extend my genuine appreciation for everything they have done in my life.

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DECLARATION

I, Hosticks Mukendwa Ndozi, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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ACRONYMS

ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
Ca	Calcium
CF	Crude Fibre
CP	Crude Protein
CV	Coefficient of Variation
DM	Dry Matter
DVS	Directorate of Veterinary Services
EE	Ether Extract
GIZ	Gesellschaft für Internationale Zusammenarbeit
GPS	Geographical Positioning System
HCL	Hydrochloric acid
HNO3	Nitric acid
INDF	Indigestible Neutral Detergent Fibre
MAWF	Ministry of Agriculture, Water and Forestry
ME	Metabolisable Energy
N	Nitrogen
NaOH	Sodium hydroxide
NDF	Neutral Detergent Fibre
NFE	Nitrogen Free Extracts
NSC	Non Structural Carbohydrate
OM	Organic Matter

P	Phosphorus
pdNDF	Potentially digestible portion of NDF
RCD	Randomized Complete Design
SE	Standard Error
SFM	Sunflower meal
SPSS	Statistical Package for Social Sciences
TDN	Total Digestible Nutrients
WSC	Water soluble carbohydrates

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CHAPTER 1

GENERAL INTRODUCTION

1.1. Background of the study

Bush encroachment refers to an accumulation of several indigenous bush species due to inappropriate management of rangelands resulting in imbalance of grass ratio to bushes (Harmse *et al*., 2016). Bush encroachment has caused serious land degradation to Namibian rangelands in both commercial and communal farming systems (Kgosikoma and Mogotsi, 2013) and has also reduced carrying capacity which results in severe economic losses for Namibia, in both the commercial (freehold) and communal (non-freehold) farming areas (de Klerk, 2004). In Namibia, livestock predominantly depend on rangeland for grazing and browsing.

Frequent droughts in Namibia decrease the available grazing and reduce the quality of grazing materials particularly as reflected in the protein and digestible energy content. There is an abundance of encroacher bush species, but these cannot be efficiently utilized by livestock. The most abundant encroacher bushes in Namibian rangelands are *Senegalia mellifera, Dichrostachys cinerea, Catophractes alexandrii* and *Vachellia reficiens* (de Klerk, 2004; GIZ, 2016). Namibian farmers have adopted a way of feeding livestock with bush pellets, but these have high fibre content which decreases the feed intake (GIZ, 2016). Feeding bush products alone does not necessarily provide enough nutrients to livestock due to the high fibre content (Herman *et al*., 2016). High amounts of lignocellulose as a result of high fibre content reduces the ruminal degradation of some components of carbohydrates (Borrero-López *et al.*, 2018).

The use of different treatments, which degrade fibre, increases animal productivity when compared to untreated bush feeds (Verma *et al*., 2006). Physical, biological and chemical treatments have been used across the globe with the aim of improving the degradation of fibre in feeds (Buxton and Redfearn, 2018). Alkali treatment of orchard grass frequently affected the composition of forage and *in situ* disappearance linearly in the range of 0 to 8% (Canale *et al*., 1990). Alkali solubilizes hemicellulose and breaks the ester bonds between the hemicelluloses and lignin (Canale *et al*., 1990). Rice straw treated with urea at 3% and lime at 4% for three weeks had increased degradability (Sarnklong *et al*., 2010). Urea treatment of straw increased feed intake, palatability and digestibility (Ju *et al*., 2013). Urea treatment at 4% affected the chemical composition (CP, ADL and ME) of field pea straw and significantly reduced NDF and ADF content of the feed (Wamatu *et al*., 2017). Al-Suwaiegh and Al-Yousef (2016) reported a decrease in NDF and ADF concentration when palm leaves were treated with urea at 4%. In addition, NDF and ADF concentration were reported to decrease linearly in sugarcane bagasse when increasing the levels of sodium hydroxide (Murta *et al*., 2011).

The addition of exogenous fibrolytic enzymes to concentrate feeds for one month before feeding showed an increase in milk production and diet digestion by dairy cows (Habib *et al*., 2016). Sujani and Seresinhe (2015) reported that the application of fibrolytic enzymes at forage harvesting improved the crude protein digestibility, crude protein intake, dry matter intake and dry matter digestibility of Bermuda grass hay. Sheikh *et al*. (2017) investigated the digestibility parameters of Ossimi sheep fed with enzyme-treated rice straw and the results showed a significant increment in straw digestibility. The use of fibrolytic enzymes (2%) in total mixed rations did not have impacts on dry matter intake and digestibility (Pinos-Rodr´ıguez et al., 2008).

There are several reasons why farmers opt not to use the existing methods in improving the utilization of agricultural by-products for animal feeds including economic, practical exposure, physical and environmental conditions (Olafadehan, 2013). Production of bush-based animal feed is an economic decision which entails the right treatment to use depending on the cost effectiveness of such treatment. Therefore this study investigated chemical treatment options for the abundantly available encroacher bushes to be used as a feed resource for livestock. The expected outcome for this study was to ensure a reduction in fibre content due to chemical treatments and enhance palatability of bush-based animal feeds, which may ultimately lead to increased live weight gains per hectare and profitability.

Palatability can be explained as the summation of taste, odor, appearance, texture and temperature of a feedstuff which determine its degree of acceptance (Jurgens *et al*., 2012). Palatability usually designates those characteristics of a feed that invoke a sensory response in the animal, and is considered to be the corollary of the animal's appetite for the feed (Gunasekaran *et al*., 2014). The voluntary intake of the feed is defined as the amount of dry matter consumed each day when livestock are offered excess feeds (Mehra *et al*., 2001). Other ancillary indicators to collect data on palatability includes the differential palatability of forage which is done through observing how animals are grazing on a daily basis (Hussain and Durrani, 2009). Palatability depends on the choice and preference that the animal has for the particular feed that is provided to it at a specific time. Moreover, palatability of the feed is affected by hairiness, cuticle thickness, odor, waxiness and thorns; and feed consumption is higher when it is highly palatable (Jurgens *et al*., 2012).

1.2. Statement of the problem

The production of bush-based animal feed includes use of branches less than 2 cm (MAWF *et al*., 2017) in diameter and these branches have cellulose, hemicellulose and lignin which increases fibre content in bush feeds. High fibre content in feeds negatively affects the fermentation of organic matter, microbes in ruminants, voluntary intake, reduces palatability and digestibility of feed (Buxton and Redfearn, 1997). The digestion of plant cell wall becomes impossible due to high amount of cellulose, hemicellulose and lignin materials, since lignin renders cell walls biologically and chemically resistant (McDonald *et al*., 2010).

Furthermore, high fibre concentration reduces the passage rate of the ingested feeds in livestock which negatively affects digestibility (Lindberg, 2014). Even though fibre is necessary, animals take more time in chewing and breaking the insoluble fibre into smaller size in order to meet the fermentation requirements of the ruminal microbes. High fibre content decreases the surface area of the alimentary tract that could be used by some of the essential components of the feed. Although commercial farmers in Namibia utilize bush-based feed for livestock (GIZ, 2016), the efficacy of the treatment methods used has not been scientifically verified which could have detrimental effects on animal health and productivity. Therefore, the use of chemical treatments in bush-based animal feeds should be published in international journals in order to provide extensive knowledge on how these chemicals are used and for farmers implement these methods. This study therefore investigated several treatment options for producing bush-based feed using *Senegalia mellifera* and the palatability of the feed so produced. *Senegalia mellifera* and *Catophractes alexandrii* were selected for this study because they are the most dominant encroacher bushes in central Namibia and their nutritional composition is higher than for most of the other encroacher species (GIZ, 2016; Marius, 2016).

1.3. Research objective

The overall objective of this study was to determine the chemical composition of *Senegalia mellifera* and *Catophractes alexandrii* and the effects of chemical treatment on chemical composition and palatability of bush-based animal feeds.

1.3.1 Specific objectives

1. To determine the chemical composition of milled twigs and leaves of *Senegalia mellifera* and *Catophractes alexandrii*.
2. To determine the nutrient composition of formulated diets from *Senegalia mellifera*, developed using seven different treatments (control = [CTRL], 4% urea = [urea4], 6% urea = [urea6], 8% urea = [urea8], 4% NaOH = [NaOH4], 6% NaOH = [NaOH6] and 8% NaOH = [NaOH8]).
3. To determine the palatability of the feeds developed from treated *Senegalia mellifera* encroacher bush.

1.4. Research hypothesis

H_{01}: There are no significant differences in chemical composition of milled pods, twigs and leaves from *S. mellifera* and *C. alexandrii.*

H_{02}: There are no significant differences in the nutrient composition of formulated bush feed from *Senegalia mellifera* using the seven different treatments (CTRL, urea4, urea6, urea8, NaOH4, NaOH6 and NaOH8).

H_{03}: There are no significant differences in palatability of the four types of feeds formulated from treated (CNTRL, 4% urea, 4%NaOH and, 4% enzyme) *Senegalia mellifera*.

1.5. Significance of the study

Feeding livestock with high amounts of fibre has negative impacts on the digestive physiology of animals, therefore there is a need to carry out experiments to determine effective ways of minimizing fibre content of bush-based animal feeds. The results of this study could benefit farmers who frequently grapple with scarcity of feed especially in drought periods. The results of this study could provide entrepreneurial opportunities through production and sale of bush-based feed. Indirectly, the commercial exploitation of encroacher bush for feed production would increase carrying capacity, hence boosting livestock productivity.

1.6. Limitations

Financial and time constraints restricted the number of treatment combinations that could be investigated. A literature search helped in reducing the number of treatments to be investigated to those with a high likelihood of success. However, the best treatment options identified may not necessarily be the most appealing to the end users and may not be the most cost effective. The research focussed on *S. mellifera* and *C. alexandrii*, yet encroacher bush species differ by agro-ecological zone, hence the results may be of limited relevance to producers in other regions.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Bush encroachment is a worrisome form of land degradation which remains a challenge to most scientists due to its negative impacts on biodiversity and agricultural productivity. Bush encroachment is a term that describes the incursion or thickening of the whole landscape by few of the several indigenous bush species which speculatively flourish leading to the unsustainable management of rangeland resources (Kgosikoma, 2012). Bush species are opportunistically expanding in density and area coverage due to some factors including environmental variability and human induced forces (Rothauge, 2017). Moreover, some of the drivers to bush encroachment are rainfall fluctuations, overgrazing and fire suppression. The main encroacher species in Namibia according to Rothauge (2017), include *Vachellia luederitzii* (formerly *Acacia luederitzii*), *Senegalia mellifera* (formerly *Acacia mellifera*), *Vachellia reficiens* (formerly *Acacia reficiens*), *Colophospermum mopane*, *Dichrostachys cinerea*, *Rhigozum trichotomum*, *Terminalia prunioides*, *Terminalia sericea* and *Catophractes alexandrii* (Figure 2.1).

In semi-arid savannahs of southern Africa, about 20% of rangelands are covered by shrubs while in the past two decades grasses have decreased up to 30% (Birhane *et al*., 2017). According to Elias and Tischew (2016) the taxonomic groups which include ungulates, rodents, lizards, birds and carnivores have shown a shift in their community structure due to increased bush cover in most savannahs. High dependence of mammalian species on grassland resources and vegetation structure make the taxonomic levels of mammals vulnerable to bush encroachment (Harmse *et al*., 2016).

Moreover, mammals use the landscape in numerous ways which is influenced by the alteration in vegetation structure and composition as a result of bush encroachment.

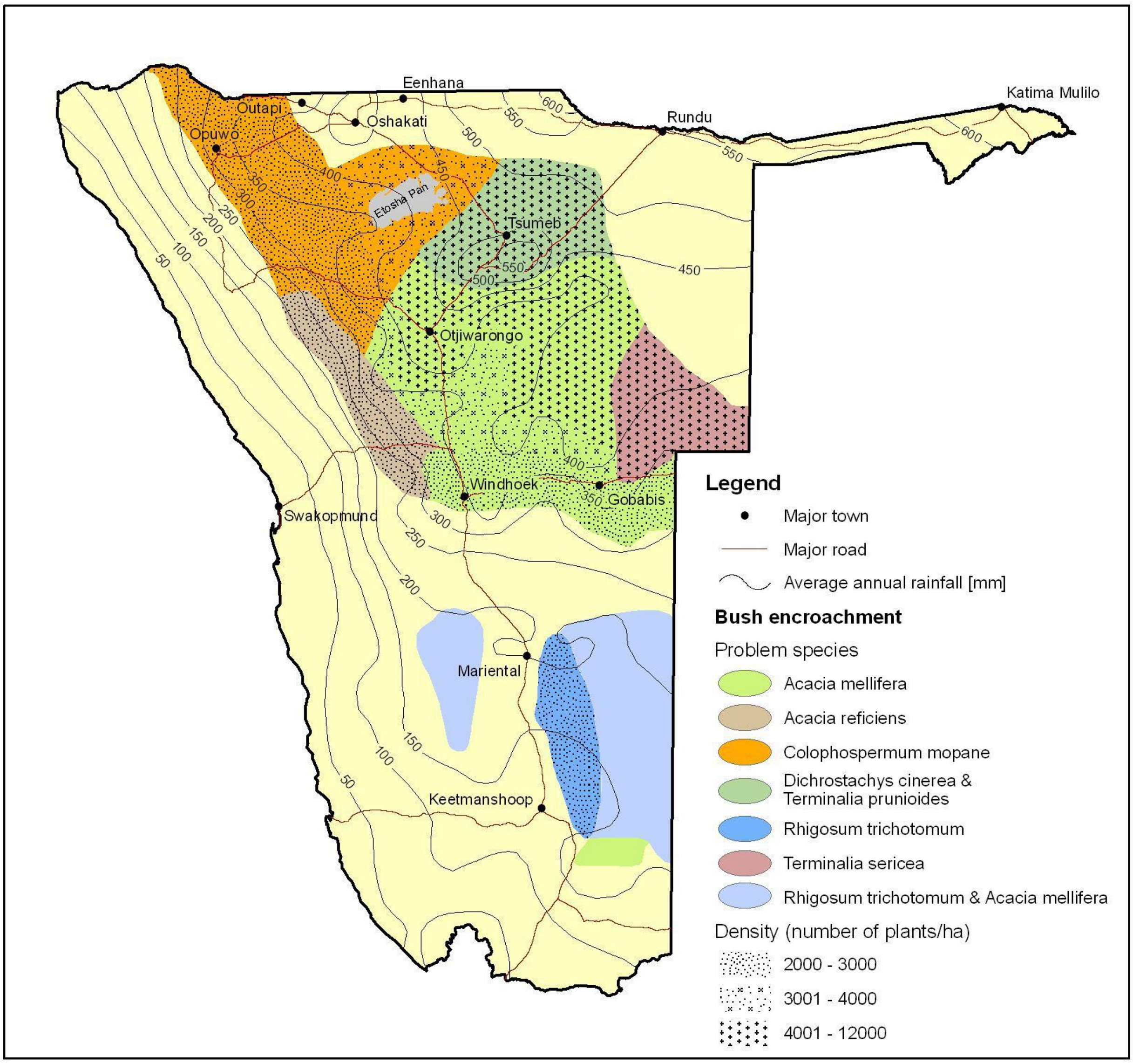

Figure 2.1. Bush encroached areas and problem species in Namibia (Source: Stehn, 2008).

Suppression of herbaceous species occurs with the increase in woody species which decreases the availability of palatable grasses and other herbaceous plants which provide foraging materials to many savannah mammals (Haussmann *et al*., 2016). *Senegalia mellifera* is one of the main bush encroacher species in African savannahs

(Hunziker *et al.,* 2017). Bush encroacher species such as *Senegalia mellifera* have extensive root systems that can able to extend into the soil profile which increases the competition in the absorption of water and other minerals from the soil (Huziker *et al*., 2017). Chemical methods by the application of arboricide and physical methods such hand clearing with axes, are some of the common methods of clearing S*enegalia mellifera* (Lohmann *et al*., 2014).

Due to frequent occurrence of droughts in Namibia, a potential of converting bush species into animal feed exists, which may help mitigate the effects of drought in both communal and commercial farming areas (Lukomska *et al*., 2014). The utilization of bushes as animal feeds during droughts is, however, hindered by excessive fibre content which tends to reduce feed intake and digestibility of the feeds. High amount of fibre content caused longer chewing and rumination time when ruminant dairy cows were fed with hay (Tafaj *et al*., 2005).

Fibre content in low nutrient feeds can be reduced by mechanical, chemical and biological treatments. The main focus of this study was to use chemical treatments (sodium hydroxide and urea) and biological treatment using fibrolytic enzymes to improve the nutritive value of bush-based feed. Several Namibian farmers from both commercial and communal farming systems have embarked on turning encroacher bush species into a desirable bush-based animal feed (GIZ, 2016). Bush-based feed may not only serve as drought feed, but also as a supplementary feed on a yearly basis.

2.2. General overview of bush encroachment in Namibia

Bush encroachment reduces agricultural outputs predominantly livestock production and it also affects biological diversity. In Namibia about 26-30 million hectares were estimated to be bush-encroached (GIZ, 2015). The causes of bush encroachment are not well understood are varied and there is no general consensus on the subject. Low rainfall is hypothesized to be a main contributor to bush encroachment. Continuous occurrence of low rainfall for multiple years in rangelands allows the increase in woody vegetation cover most especially encroacher bush species such as *Senegalia mellifera* (Joubert *et al*., 2008). In addition, high amounts of soil moisture in areas where there is limited competition from grasses, promotes the survival rate and growth of woody plant seedlings into bush thickets.

Suppression of fire in the veld is also thought to contribute to bush encroachment. Kgosikoma *et al*. (2012a) stated that fire remains a management tool that reduces woody species. Some pastoralists and ecologists argue that the proliferation of woody vegetation is a result of suppressed burning of fire in savannah ecosystem. Furthermore, burning of fire in savannah ecosystem helps in destroying the shrubs and juvenile trees which suppress the woody plant growth and also prevent their development into mature woody plants (Mphinyane *et al*., 2011). Furthermore, replacement of mega browsers with grazers, leads to overgrazing.

There are ongoing projects that seek to control bush encroacher species through the application of physical, biological and chemical methods so as to promote a long-term rehabilitation of the rangelands (Ludwig *et al*., 2016). The introduction of bush harvesting in Namibia does not necessarily remove the entire bush encroacher species at a particular area, but rather minimizes the density of encroacher bush species on rangelands in order to promote the determined carrying capacity of the rangeland (de Klerk, 2004).

2.3. Chemical composition of *Senegalia mellifera* and *Catophractes alexandrii*

Table 2.1 shows that *Senegalia mellifera* leaves and pods had more desirable chemical composition compared to *C. alexandrii* (Marius, 2016). High amounts of fibre content in bush encroacher species tend to reduce the digestibility and palatability of these species when used as animal feeds.

Table 2.1. Chemical composition of leaves and pods of woody plant species.

Species	**DM** g/kg	**OM** g/kg DM	**Ash** g/kg DM	**CP** g/kg DM	**NDF** g/kg DM	**ADF** g/kg DM
S. mellifera	944.7	878.8	65.9	118.3	336.0	247.7
C. alexandrii	942.6	880.2	62.5	101.0	400.3	288.9

Source: Marius (2016)

The chemical composition is affected by the plant parts harvested. In the study of Marius (2006), *Senegalia mellifera* and *Catophractes alexandrii* bushes with small branches less than 2 cm in diameter were harvested in April and milled for the analysis of chemical composition (Table 2.2).

Table 2.2. Chemical composition of two encroacher bush species

Species	**Moisture** g/kg DM	**CP** g/kg DM	**NDF** g/kg DM	**ADF** g/kg DM	**Fat** g/kg DM	**Ash** g/kg DM
S. mellifera	46.6	124	556.6	471.7	26.6	53.1
C. alexandrii	66	65.3	528.6	427.9	19.2	55.1

Source: MAWF *et al*. (2017)

Table 2.3 shows the chemical composition of several foliage bush species (Osuga *et al*., 2008). The CP content range was from 149.5 g/kg DM in *S. mellifera* to 248.6 g/kg DM in *M. angolensis*. *Z. mucronata*, *S. mellifera* and *A. brevispica* had the highest NDF, ADF and ADL contents, respectively. *Senegalia mellifera* which was one of the five studied browse foliages was considered to be transitional to high nutritional quality because it contained 149.5 g/kg DM of CP and 395.4 g/kg DM of NDF (Leng, 1990). Browse foliages that have high CP content ranging from 149.5 g/kg DM to 248.6 g/kg DM provide a great motivation on the use of browse foliages as a supplementary of poor natural pastures (Osuga *et al*,. 2006). Bakshi and Wadhwa (2004) reported that fibre content that is low to moderate in browse foliage would influence the voluntary intake and digestibility of the foliages.

Table 2.3. Chemical composition of selected browse foliages from semiarid area of Kenya**.**

Species	DM g/kg	OM (g/kg DM)	CP (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)
S. mellifera	899.3	932.3	149.5	395.4	322.2
A. brevispica	893.1	970.6	236.3	417.9	255.4
B. discolor	884.4	961.0	216.3	423.0	202.6
M. angolensis	862.8	933.8	248.6	205.1	119.5
Z. mucronata	877.1	960.4	192.8	424.0	226.7

Source: Osuga *et al*. (2008).

2.4. Application of chemical treatment in low quality feeds

In tropical regions, farmers store agricultural by-products such as rice straw which are used as ruminant feeds; notwithstanding, these agricultural by-products have a low percentage of major nutrients such as protein, vitamins, minerals and nitrogen (Hassan *et al*., 2011). Straw and other agricultural by-product contain cellulose and hemicellulose which can hardly be degraded by the rumen microbes and ultimately hinders the uptake of nutrients for a satisfactory performance of livestock.

Chemical, biological and physical treatments have been used globally as a potential way of improving nutritional values of high fibrous feeds (Ma *et al*., 1990; Nisa *et al*., 2004; Pinos-Rodríguez *et al*., 2008; Abdal-Hafiz, 2016). The chemical treatments used include use of urea, sodium hydroxide and calcium oxide; the biological treatments include use of fibrolytic enzymes. Exposure of straws to alkali treatments hydrolyses the ester linkages between lignin and the cell wall polysaccharides (celluloses and hemicelluloses), leading to greater availability of the carbohydrates to the microorganisms in the rumen (McDonald, 2011). To our knowledge, there is paucity of information on the effectiveness of chemical treatments in reducing the lignin content of bush-based feed and hence the need for investigation of this aspect.

2.4.1. The use of urea in improving animal feeds

Urea treatment of fermented wheat straw showed an increase in the availability of structural carbohydrates through the change in the cell wall structure for ruminal microbial fermentation (Sarwar *et al*., 2004). In addition, when ruminant were fed with sugarcane residues treated with urea, the digestibility of dry matter and organic matter was increased (Hassan *et al.,* 2011). The effective degradation of cell surface structure by ruminal microbes differs depending on the thickness of the cell wall and the presence of lignin (Nisa *et al*., 2004; Hassan *et al.*, 2011). Treatment of cocoa pods with 5% urea gave the best digestibility compared to other treatments (Anggraeni, 2017). Furthermore, urea treatment improved the nutritional value and intake of cocoa pod. Using 5% urea was recommended as safer because there is no production of ammonia which might negatively affect the microbes.

The chemical composition of untreated paddy straw, urea molasses treated paddy straw and urea molasses plus enzyme treated paddy straw is shown on Table 2.4. A

Treatment of urea molasses reduced neutral detergent fibre and acid detergent fibre (ADF) content of rice straw (Sheikh, 2007; James, 2009). Additionally, there was improvement in crude protein (Sheikh, 2017). The chemical bonds between cellulose, hemicellulose and lignin are easily broken by urea treatments providing a conducive environment for rumen microbes to breakdown the structural carbohydrates, which enhances degradability and this was associated with higher feeding intake (Hassan *et al.*, 2011).

Table 2.4. Chemical composition of untreated rice straw, urea-molasses treated rice straw with and without fibrolytic enzymes.

Chemical composition (g/kg DM)	Un-treated Paddy straw	Urea molasses treated Paddy straw	Urea molasses + Enzyme treated paddy straw
DM	940.0	52.34	78.98
CP	35.0	64.0	64.8
EE	15.0	15.6	15.5
NDF	849	739	719
ADF	613.0	539	518.7
Ash	49.0	61.2	49.8
Ca	4.1	4.3	4.2
P	2.1	2.2	2.2

Source: Sheikh *et al.* (2017).

Lambs that were fed on singular urea treated straw had a higher dry matter intake compared to those fed untreated straw. Increase in dry matter intake of straw by 68% was observed when urea was added to untreated straw (Gatahum, 2013). The addition of 100 g of urea per kilogram of block was recommended, as this stimulated the microbial activities through maintaining the optimum level of ammonia in the rumen (Dona *et al*., 2013).

2.4.2. Application of sodium hydroxide (NaOH) to low quality feeds

Several sodium hydroxide (NaOH) treatment methods have been developed in most of the tropical regions aiming at improving the use of crop residues for ruminant feeding. Treatment with NaOH increases the degradability and palatability of low quality crop residues. Melingasuk *et al*. (2016) reported a 27% increase in DM intake of sorghum residue treated with NaOH. Treating corn cobs with NaOH at 4% showed an increase in DM digestibility by 11.2% in comparison with the untreated control (Klopfenstein *et al*., 1972). In a similar study, it was found that the in-vitro DM digestibility increased by 9.7% while in the in-vivo digestibility only increased by 2.5%.

The increase in daily gain, feed efficiency and dry matter intake was reported more in studies where 3% of NaOH was mixed with 1% $Ca(OH)_2$ than using 4% NaOH as an individual treatment in improving crop residues (Bindar *et al*., 2012). Some of the drawbacks associated with the application of NaOH treatments is that the chemical is not readily available for communal farmers and may be costly to buy (Sarnklong *et al*., 2010). The use of NaOH treatments in production of animal feeds pollutes the environment, which negatively affects the biological diversity (Melingasuk *et al*., 2016).

2.4.3. Application of fibrolytic enzymes to low quality feeds

The readily available fibrolytic enzymes for degrading fibre include cellulases and xylanases (Avellaneda *et al.*, 2009). One of the limitations associated with low degradation of fibre when using enzymes is that some of these enzymes might not have the ability of breaking the esterified bonds within lignin-carbohydrate complexes

(Morones *et al*., 2017). The increase in numbers of ruminal fibrolytic microbes through the direct feeding of enzymes enhances the microbial colonization (Gado *et al.,* 2009; Pinos-Rodríguez *et al,* 2008). The inclusion of enzyme treatment in feeding of ruminants have been reported to improve the feed intake as well as increasing palatability (Almaraz *et al*, 2010).

A study done by Salem *et al*. (2012) showed an increase of 46% DM intake and 11% digestibility when exogenous enzymes were added to *Atriplex halimus* foliage fed to sheep. Other studies (Pinos-Rodríguez *et al.*, 2008; Avellaneda *et al.*, 2009; Sujani and Seresinhe, 2015) have reported an increase in the intake of dry matter predominantly in the digestibility of fiber, when exogenous enzymes were added. Fibrolytic enzymes reduce the digesta viscosity which helps in altering the fermentation within rumen system and also enhance the rumen microbes' ability to colonize the cell wall of the plant (Pinos-Rodríguez *et al.*, 2008; Sujani and Seresinhe, 2015).

The application of exogenous fibrolytic enzymes that are rich in xylanolytic activity was suggested by Pinos-Rodríguez *et al.* (2008) to increase the apparent digestibility of crude protein in addition to the apparent digestibility of NDF in alfalfa hay. According to Sheikh *et al*. (2017) the findings showed that there was a reduction in NDF and ADF content while CP content increased when rice straw mixed with urea molasses and fibrolytic enzyme treatment were fed to Sheep.

2.5. Palatability of feed and its determinants in ruminants

Palatability plays a significant role in the nutrition of animals as it may influence an animal's acceptance or rejection of forage. Palatability describes the characteristic of the feed which, together with the gustatory, olfactory and visual perception, determines the choice by the animal (Mertens, 1996; Aguiar *et al*., 2015). Palatability was further explained as the interrelationship between taste and the effects that result from feed intake, as influenced by the chemical characteristics of the feed, by the nutritional status of the animal and by the past experiences at the time of supply of the feed (Rolls, 1986; Provenza *et al*., 1995; Provenza, 1996).

Animal factors such as phase of pregnancy, hunger of animal, general health and differential preference for forage species affects the palatability of the plants or feeds. Furthermore, chemical nature, fibre content, presence of tannins, seasonal availability of the plant, morphology, phenology, degree of maturity and stage of growth are some of the plant factors that affect palatability (Karak *et al*., 2012). Ruminant animals such as cattle, sheep and goats differ in degrees of sensitivity to palatability factors when a choice of feed is offered (Marten, 1978).

The oropharyngeal senses possessed by the animal such as taste, texture and odour are the major determinates for palatability of the forage. Ruminants most precisely cattle, sheep and goats possess different degrees of selection which increases acceptance and palatability (Baleseng and Kgosikoma, 2016). Palatability depends on the availability of the plant species to the animals and palatability of the plants varies with altitude and location where it grows throughout the year (Khan and Hussain, 2012). The variation is mainly determined by some of the environmental factors such as rainfall and soil fertility. Furthermore, palatability of forage species to the animals is dependent on the climate and phenological stages of the plant (Habib *et al*., 2016).

The palatability of highly lignified forages can be improved by using different approaches such as moistening the feed with chemicals and pelleting (Karak *et al*., 2012). The preference patterns for a feed depends on the different physical characteristics that are exposed such as the spines, waxy layers on the leaf structure and taste presence of fibrous material of the feed. Flavor in small ruminants plays a crucial role in improving the choice or preference or voluntary feeding intake as demonstrated in some of the studies conducted on sheep and goats (Distel *et al*., 1994).

Physical characteristics of any given forage remains an important aspect associated with low palatability, for example, milling of shrub should include leaves attached to twigs in order to increase the intake, than offering plant parts without leaves and twigs (Baleseng and Kgosikoma, 2016).

The nutrition state of the animal, chemical and physical characteristics of the feed influence palatability of the feed. The presence of anti-nutritional compounds in browse species which includes tannins leads to variation in feed intake of ruminants which influence the selection of diets (Khan and Hussain, 2012; Rapisarda *et al*., 2012). Grasses and browsable plants which are rich in water soluble carbohydrates (WSC) have a sweet taste which is associated with the choice and preference of the feeds for most livestock (Saidi *et al*., 2013; Baleseng & Kgosikoma, 2016).

Almost all grazing ruminants have the ability of differentiating the grasses that exist in the wild and the grasses that are cultivated. Ruminants have the ability of selecting grasses that meet their nutritional requirements and avoiding those grasses that possess toxic compounds through the involvement of sensorial perception and the feedback of post-ingestive effects (Hussain and Durrani, 2009). Growing animals use post-ingestive feedback effects to select suitable forage through the process of learning from their mother and other group members during grazing; this enables the young animals to gain necessary experience on which forage to ingest (Saidi *et al*., 2013).

2.5.1. Palatability assessment

Choice and selection in ruminants is influenced by the past experience which is based on the feedback preference, which is influenced by factors such as chemical characteristics and liking the flavor of the feed (Meier *et al*., 2012). Different roles of smell and taste on the feed is one of the other factors that remains a challenge in measuring the palatability of the forage. Ideally, palatability assessment should not be influenced by the earlier consumption of feeds or intake as a result of post-ingestive processes (Meier *et al*., 2012). In order to minimize risks of confounding palatability with post-ingestive effects, the voluntary feed intake should be recorded during the first time when the feed is exposed to the animal (Karak *et al*., 2012).

2.6. Plant type and constituents

Generally, grasses and legumes as animal forages differ in terms of their fibre digestibility. Grasses are not easily digested by animals because they have a high amount of fibre content, while legumes are easily digested because of their low fibre content. In contrast, the fibre in legume plants species are more lignified and the digestibility is lower than the fibre present in grass species. High fibre content and lower cell soluble concentrations are the major factors that decrease the digestibility of forages in nature (McDonald *et al*., 2011). Feed from encroacher bush may have lower digestibility because of the high fiber concentration, particularly when harvested late in the growing season; this aspect requires investigation for different encroacher bush species.

The rate at which digestion takes place depends on the particle size: large particles are not digested as fast as small particles, this is because small particle have more surface area that is exposed relative to the volume of tissues (Paulson *et al*., 2008). All ruminant animals have the ability to reduce the particle size of the forage through the process of regurgitation. Besides, ruminants spend more time when chewing mature grasses and legumes than when chewing the immature ones and spend less time when regurgitating and chewing legumes than grasses. Legume particles in the rumen are often cuboidal, whereas grass particles are elongated and slender (McDonald *et al*., 2011). Filamentous grass particles are usually slower to pass from the rumen than the cuboidal fragments of legumes.

Lignin is necessary to provide mechanical support for stems and leaf blades and to impart strength and rigidity to plant walls (Buxton *et al*., 1996). Lignin and lignin like compounds along with other cell wall components provide resistance to diseases, insects, cold temperatures and other biotic and abiotic stresses. Thus practical limits exist as to how much lignin and other cell wall constituents can be reduced in forages to grow and survive under field environments. Legume forages such as alfalfa selected for low lignin concentration grew less vigorously and had lower field survival than alfalfa selected for high lignin concentration. Likewise, switch grasses selected for three cycles of high digestibility had lower field survival than the base population (McDonald *et al*., 2011).

2.7. The effect of neutral detergent fibre (NDF) on dry matter intake

Neutral detergent fibre (NDF) has been proposed as the best single chemical predictor of the voluntary dry matter intake in ruminants (Waldo, 1986). None the less the filling effects of NDF are influenced by the initial size of particles, particle fragility and rate and extent of fibre digestion (Allen, 1996). Indigestible Neutral Detergent Fibre

(INDF) is the most essential aspect that influence the total diet organic matter digestibility (Nousiainen *et al*., 2004). Potentially digestible portion of NDF (pdNDF) is directly related to ruminal digestibility of NDF in most of the C4 forages (Harper and McNeill, 2015).

Dry matter intake can be affected by great variation in pdNDF of the feed (Harper and McNeill, 2015), hence it is recommended that during diet formulation and forage evaluation it is important to have a precise estimation of pdNDF. Soufizadeh *et al*. (2018) explained that as the plant mature, the level of NDF increases due to lignification which tend to decrease digestibility. Plant maturity is a primary factor that influences digestibility (Harper and McNeill, 2015). Other factors that affect the quality and quantity of pdNDF include plant genetics and the environment where the plant is growing.

2.8. Lignin

Lignin is the second most abundant organic polymer on earth consisting of 15-25% of the woody plants expressed in dry weight (Borrero-López *et al*., 2018). Lignin refers to the anti-quality component of the feed that is made up of complex organic polymers which play an essential role in supporting plants vascular tissues. This class of anti-quality components is commonly found in woody plants and it is recognized as a highly polymerized (Moore and Jung, 2001). Lignin in most cases impregnate the cellulose walls of the woody plant, a process termed as lignification. Lignin is the main hinderance to fiber digestion (Van Soest, 1987) because it restricts enzyme access to cellulose and xylan and reversibly or irreversibly adsorbs enzymes, which lowers rates of hydrolysis of structural carbohydrates (Bansal et al., 2009; Ju et al., 2013).The negative relationship between degree of lignification and cell wall digestion in forages is well recognised (Buxton and Redfearn, 1997).

2.9. Limitations of plant parts digestion and utilization by ruminants

The main source of dietary fibre for animals is the plant cell wall. The digestive enzymes found in mammals cannot degrade the polysaccharides found within the cell wall of the plant. In order to satisfy their energy requirements, ruminants rely on rumen microbial fermentation to breakdown plant fibre. Neutral detergent fibre (NDF) measures the amount of fibre present in a feed (Buxton and Redfearn, 1997), with the normal amount ranging between 30-80% of the organic matter (OM) in forage crops. There is a great variation in the nutritional availability of fibre to ruminants and this depends on the composition and structure of the plant cell wall (Farhat *et al*., 2018). Grasses contain more NDF than legumes hence when diets are formulated to contain an equal amount of forage DM, the total dietary NDF concentration will be higher for diets containing grasses compared to legumes (Paulson *et al*., 2008). One of the limiting factors for fibre digestibility in ruminants is the presence of lignin which is highly resistant to chemical degradation. Physical encrustation of plant fibres by lignin makes them inaccessible to enzymes that would normally digest the fibre (McDonald *et al*., 2011). Moreover, the utilization of fibre by ruminal microbes is limited by the presence of physical constraints at the cellular organization level.

2.10. Fibre concentration and its impact on digestibility

Plant stems tend to have high concentration of fibre than leaves because these plant components contain more of the structural and conducting tissues; plant stems further develop lignocellulose which serves as the protective mechanisms from abiotic and biotic factors (Buxton and Redfearn, 1997). Dry matter digestibility is reported to be higher in legumes than in grasses due to the difference in the structure of leaves and stems. As the plants reach their maturity stage, digestibility declines in plant stems faster than in leaf blades. In a comparative study of grass and legume forages for dairy cattle, Paulson *et al*. (2008) reported that in alfalfa the digestibility declined about 20g per kg per node. Generally, when plants approach their maturity stage, the concentration of fibre increases mainly in leaves and stems. Additionally, the digestibility of leaf to stem reduces as plants mature because the amount of fibre concentration also increases in the total forage (Amiri and Shariff, 2012).

CHAPTER 3

DETERMINATION OF CHEMICAL COMPOSITION OF *SENEGALIA MELLIFERA* AND *CATOPHRACTES ALEXANDRII* ENCROACHER BUSH SPECIES

3.1. Introduction

Generally encroacher bush species are not known as feeds for livestock and information on their nutritional composition as bush-based animal feeds is not well documented. *Senegalia mellifera* and *Catophractes alexandrii* are some of the most dominant encroacher bush species in Namibia and their utilization as livestock feeds is a relatively new concept to science. Most of these encroacher species are mainly used for charcoal, firewood, traditional fences and other uses (GIZ, 2016). Evaluating the chemical composition of the feeds also assists in knowing the digestibility of the feeds.

Harvested bushes with twigs and small branches tend to have high amount of fibre content which is associated with low digestible OM and low CP content resulting in low nutritional value. Turning encroacher bush species into animal feeds can be important in improving the nutritional value of ruminants especially during periods of feed scarcity, but there is insufficient information on their chemical composition. Therefore, the objective of this experiment was to determine the chemical composition of milled mixed twigs and leaves of *Senegalia mellifera* and *Catophractes alexandrii*.

3.2. Materials and Methods

3.2.1. Collection of *Senegalia mellifera* and *Catophractes alexandrii* samples

Samples were obtained from *Senegalia mellifera* and *Catophractes alexandrii* at University of Namibia, Neudamm Campus farm, which is located ± 30 kilometers east of Windhoek on the B1 road to Hosea Kutako International Airport. The study site is categorized under semi-arid highland savanna with *Senegalia mellifera* as the most dominant bush species. The area has an average minimum temperature of 3°C and maximum temperature of about 29°C (Jourbert *et al.,* 2008). The mean annual rainfall on the farm is about 350 mm.

Sampling of *Senegalia mellifera* and *Catophractes alexandrii* was carried out in all nine blocks of the Neudamm Farm (Figure 3.1), and in each block five camps where selected randomly. Two transects were laid in each selected camp. A belt transect of 2m × 50m was used to collect small branches not exceeding 2 cm in diameter of *Senegalia mellifera* and *Catophractes alexandrii* within transects. Five individual *Senegalia mellifera* and *Catophractes alexandrii* bush species were collected and cut into small pieces which were later put into sampling bags. Each species was kept into a separate sampling bag which was written with species name as well as the date of collection. Geographical Positioning System (GPS) coordinates were recorded from where the plants were collected.

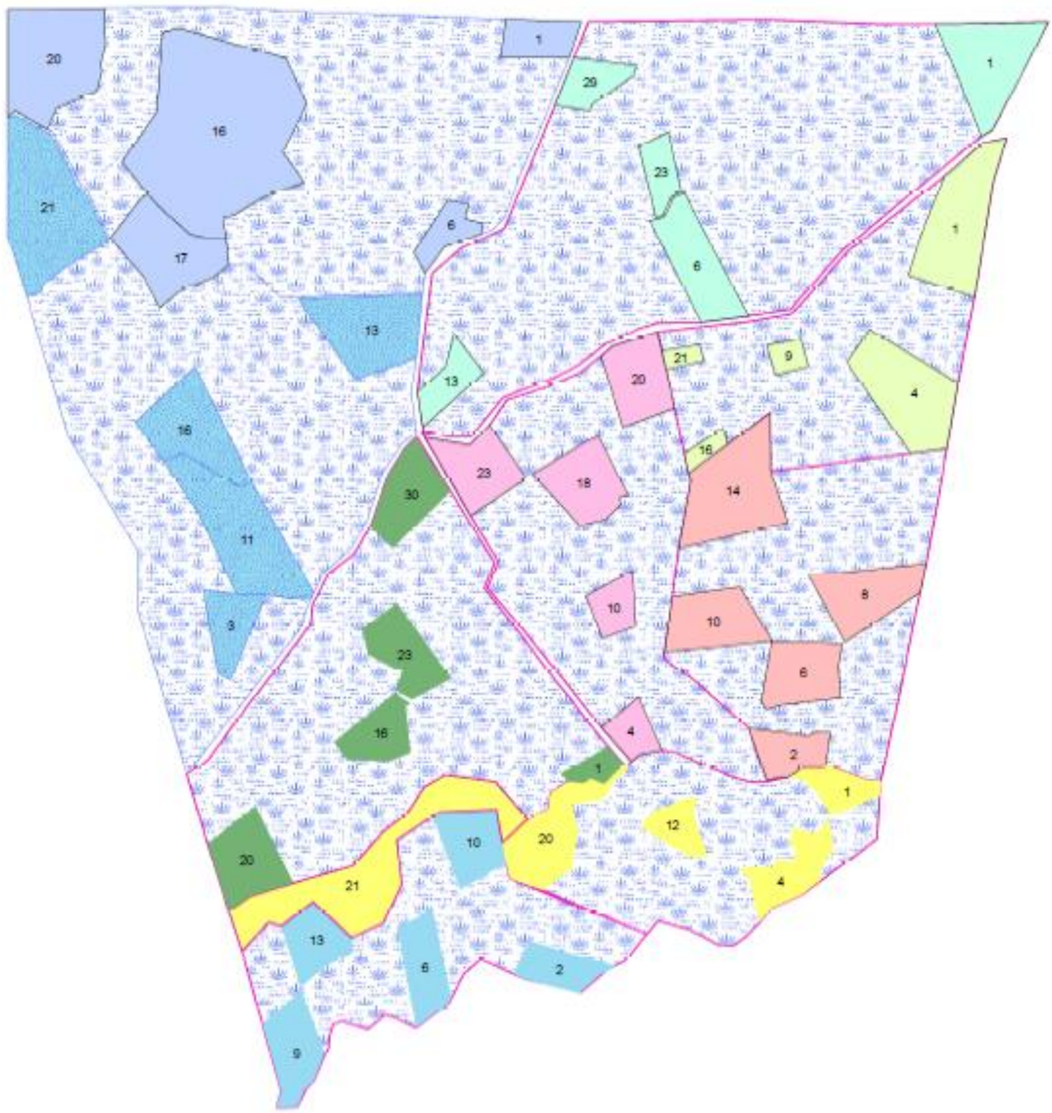

Figure 3.1. Map of Neudamm Farm showing nine different blocks where sampling of *Senegalia mellifera* and *Catophractes alexandrii* was carried out.

Fresh twigs of a diameter of 2cm or less and with leaves were harvested in March/April and shade dried at an average ambient temperature of 15.3 °C and average air humidity of 31.6% for 14 days. The dry samples of *Senegalia mellifera* and *Catophractes alexandrii* were then milled (Wiley mill) through a 2 mm sieve and taken to the laboratory for nutrient analysis.

3.2.2. Nutritional Analysis Procedures

Senegalia mellifera and *Catophractes alexandrii* samples were analyzed at the Ministry of Agriculture, Water and Forestry (MAWF) laboratory for nutritional composition (ether extract, ash, CF, NDF, ADF, ADL, Ca and P); while crude protein was analyzed at a private laboratory. Crude fiber was determined using the procedures described in Association of Official Analytical Chemists (AOAC) by Horwitz and Latimer (2005) where a FIWE Raw Fiber Extractor was used.

Milled samples (1 g) were weighed into glass crucibles. The glass crucibles with sample were inserted into the fiber extractor and 150 ml of diluted and preheated sulfuric acid (H_2SO_4) was added, three drops of antifoam agent (n-octanol) was also added and allowed to boil for half an hour. Subsequently, filtering was done for the remaining reagent and washed three times with boiled de-ionized water. Afterwards, 150 ml of NaOH was added followed by three drops of antifoam agent and allowed to boil for half an hour. Filtration was done and washed three times with boiled de-ionized water and then washed twice with acetone solution.

The remaining residue were removed carefully from the fiber extractor and taken to the oven for drying at 105 °C for six hours. After five hours, samples were removed from the oven and cooled in a desiccator for 15 minutes and weighing was done in order to obtain the crude fiber content. After weighing of dry content, dried residues were taken to a muffle furnace for ash content determination at a temperature of 550 °C for five hours, cooled for 15 minutes in desiccator and later weighed to obtain the ash (AOAC, 2006).

Crude fiber percentage was calculated using the following formula:

Crude Fiber (%) = (F1 -F2)/F0 x 100

Where F0, F1 and F2 represent the weights of sample, crude fiber plus ash content and ash, respectively.

ADF determination

The determination of ADF was carried out following the procedures described by Robertson and Van Soest (1981). A FIWE Raw Fiber Extractor was used for this particular method. Milled samples of 1 g were weighed in glass crucibles. Glass crucibles were then placed into a fiber extractor to which 100 mL of cetyltrimethylammonium bromide technical grade ($C_{19}H_{42}BrN$) and sulfuric acid (H_2SO_4) was added. Three drops of antifoam agent was also added to avoid overflowing and the samples were allowed to boil for one hour. Filtration was done immediately after boiling and remaining residues was washed three time with de-ionized water and washed two times with acetone solution. After washing with acetone, the residue was removed and oven dried at a temperature of 105 °C for five hours and cooled in a desiccator for 15 minutes. After cooling the dry residue was weighed to obtain ADF using the following formula:

ADF % = Wr/Ws x 100

Where: Wr = the weight of the residue after heating; Ws = the weight of the sample. Dried residues were taken to a muffle furnace for ash content determination at a temperature of 550 °C for five hours, cooled for 15 minutes in desiccator and later weighed to obtain the ash (AOAC, 2006).

NDF determination

NDF was determined following the procedures of Robertson and Van Soest (1981) using Neutral Detergent Solution. A FIWE Raw Fiber Extractor was used in determining NDF. Milled samples of approximately 1g was weighed in glass crucible, inserted into the extractor machine, then 100 mL of sodium borate decahydrate ($Na_2B_4O_{7.10}H_2O$), disodium ethylenediaminetetraacetate (EDTA, $C_{10}H_{14}Na_2O_8$), sodium lauryl sulfate neutral ($C_{12}H_{25}NaO_4S$), 2-ethoxyethanol (Ethylene glycol monoethyl ether, cellosolve, $C_4H_{10}O_2$), and disodium phosphate anhydrous (Na_2HPO_4) with three drops of n-octanol (antifoam agent) was added. The FIWE Raw Fiber Extractor was allowed to run for one hour while boiling the mixture. Filtration was done immediately after boiling and the residue was washed three times with de-ionized water and then washed twice with acetone solution. The residue was removed from the extractor and taken to the oven for drying at 105°C for five hours. After drying, the residue was cooled in a desiccator for 15 minutes followed by weighing and later taken to the muffle furnace for ash content determination at a temperature of 550°C for five hours, cooled for 15 minutes in desiccator and later weighed to obtain the ash (AOAC, 2006). The NDF was then calculated using a similar formula as for calculating ADF.

Ash content determination

A ground sample of approximately 2 g was weighed in a clean porcelain crucible and then placed in a drying oven at a temperature of 105°C to overnight. The residues were allowed to cool down in a desiccator for 15 minutes and then samples were weighed. The residues were dried overnight at 550 °C in muffle furnace and followed by cooling the samples in a desiccator at a room temperature for 15 minutes. The following formula was used to determine ash concentration:

%Ash = [(W_a–W_t)/(W_0–W_t)]×100

Whereas:

W_a = weight of ignited crucible + sample

W_t = Weight of oven-dry crucible

W_0 = Weight of oven-dry crucible + sampl**e**

<u>Moisture determination</u>

Moisture content was determined according to AOAC (2002). A ground sample of approximately 2 g was weighed in clean crucible and then placed in a drying oven at a temperature of 105°C to overnight. Crucibles were removed from the oven after 24 hours and were allowed to cool in a desiccator for 15 minutes. After cooling the samples, moist free sample and crucible were weighed in order to determine the moisture content by using the following formula:

% Moisture = 100 x (W_2 – W_3)/(W_2 – W_1)

Where: W_1 = the weight of the dish without lid; W_2 = weight of the sample plus dish; W_3 = weight of the sample plus the dish after drying (AgriLASA, 1993).

% DM = 100 – Moisture %

<u>Ether Extract determination</u>

Ether extract content was determined using a Solvent Extractor Machine (VELP SICIENTIFICA). Milled samples of 3g were weighed in the extraction thimbles and then taken to the Solvent Extractor Machine where they were hooked. Clean beakers were weighed which had stones in each of them and 60 ml of petroleum was added, they were then put in the extractor in which the extraction thimbles were immersed.

The knot was moved to level one (immersion), afterwards the tap was opened for condensation and allowed to boil at temperature of 110°C for one hour, and sequentially the knot was moved to the second level which washed for one hour. After one hour of washing, the knot was finally moved to level three (recover) for the last one hour of boiling. A calm boiling process was maintained through the addition of boiling stone in the beakers. After the last process which was boiling, the beakers with recovered samples were gently removed and placed in the conventional oven at a temperature of 105°C to allow drying for 30 minutes. The beakers were removed from the oven and placed in a desiccator to allow cooling for 30 minutes and then weighed in order to determine fat content using the following formula:

Crude fat (%) = MFR – MF / m × 100

Where: m, MF and MFR represents the mass of sample used, mass of flask and mass of flask with extracted residue, respectively, which are all represented in grams (g) (AgriLASA, 1993; AOAC, 2006).

Determination of phosphorus and calcium

The concentration of phosphorus and calcium for both *Senegalia mellifera* and *Catophractes alexandrii* was determined by following the procedures of dry ashing. Samples from the bush species were digested using 6 M of hydrochloric acid (HCL) and 6 M of nitric acid (HNO_3) reagents. Porcelain crucibles were used in drying bush samples (2 g) for six hours and later ashed into a muffle furnace at 550°C for 5 hours and allowed to cool down in a desiccator for 30 minutes. After cooling the samples in a desiccator, samples were heated again in 5 cm^3 of 6 M of HCL for 2-3 hours in order to dissolve them. After heating, samples were allowed to cool down then 6 M of nitric acid (HNO_3) was added. Crucibles were observed and removed when the solution started boiling and they were filtered immediately through the filter paper into 100 ml volumetric flasks. De-ionized water was used to wash the filter paper three times and the solution was mixed thoroughly and minerals were analysed using ICP Spectrometer (icap 6000 series).

3.3. Statistical analysis

Chemical composition data (DM, NDF, ADF, CF, fat, organic matter, ash) of *Senegalia mellifera* and *Catophractes alexandrii* were subjected to the analysis of variance procedure of Statistical Analysis System (SAS, 2008) according to a randomized complete block design (RCBD). The model included effects of block and encroacher bush species. Calcium showed a non-constant variance and a logarithm transformation brought about normality. Least squares means for Ca were obtained through back transformation and the standard errors were estimated from the untransformed data. Various transformations were attempted for phosphorous, but were not helpful and hence we used a non-parametric analysis. Effects were considered significant at $P < 0.05$. A trend was recognized at $0.05 \leq P \leq 0.1$. The statistical model was:

$$y_{ijk} = \mu + \tau_i + \beta_j + \varepsilon_{ijk}$$

Where: y_{ijk} = the k^{th} observation of species i in block j

μ = the overall mean

τ_i = the effect of species i

β_j = the fixed effect of block j

ε_{ijk} = random error

3.4. Results

3.4.1. Chemical composition of *Senegalia mellifera* and *Catophractes alexandrii*

The chemical composition of untreated *S. mellifera* and *C. alexandrii* differed (P < .05) (Table 3.1). *Senegalia mellifera* had a higher (P < .05) concentration of CP, NDF, ash, Ca and CF than *C. alexandrii*. Model fit was good ($R^2 \geq 0.70$) for all variables except for ADF and CF. Samples showed the highest coefficient of variation (CV) for ash and Ca; lowest CV was for OM and DM. Results of the Krusk-Wallis non-parametric test are shown in Table 3.2. *Catophractes alexandrii* had a higher concentration (P < .05) of phosphorous than *S. mellifera* (Table 3.2).

Table 3.1. Chemical composition (g/kg DM) of *Senegalia mellifera* and *Catophractes alexandrii* samples*

Variables	*S. mellifera*	*C. alexandrii*	p-value	S.E	CV	R^2
DM	940.93	921.24	<.0001	0.13	0.571	0.854
Ash	52.27	39.76	< .0001	0.11	10.30	0.727
OM	947.73	960.24	<.0001	0.11	0.475	0.743
CP	147.42	60.92	<.0001	0.37	6.518	0.980
CF	444.98	409.03	<.0001	0.48	4.75	0.612
NDF	653.63	606.10	<.0001	0.41	2.75	0.773
ADF	483.82	498.34	0.0028	0.31	2.69	0.487
EE	14.22	12.30	<.0001	0.02	6.470	0.815

Calcium	12.72	7.24	<.0001	0.036	15.47	0.84

*DM = Dry Matter; OM = Organic Matter; Moisture; CP = Crude Protein; CF = Crude Fibre; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; EE = Ether Extract; s.e = standard error; CV = coefficient of variation (%).

Table 3.2. Wilcoxon scores (rank sums) used in the Krusk-Wallis non-parametric test for phosphorus (P) concentration (% DM) of *Senegalia mellifera* and *Catophractes alexandrii*

Species	**n**	**Sum of scores**	**Mean scores**
Senegalia mellifera	18	198.0	11.0
Catophractes alexandrii	18	468.0	26.0

3.5. Discussion

Encroacher bush species examined in this study cause serious land degradation in form of bush encroachment, but these bushes have potential to be used as an alternative fodder for livestock. Since the bush samples from *Senegalia mellifera* and *Catophractes alexandrii* used in this study were shade dried at an average temperature of 15.3 °C and relative humidity of 31.6%, their dry matter was expectedly higher and differed significantly as shown in Table 3.1. The hypothesis of similarity in the chemical composition of milled pods, twigs and leaves from *S. mellifera* and *C. alexandri* was rejected.

Crude protein was reported to be significantly different (P <0.05) in both *S. mellifera* and *C. alexandrii*. The CP content of *S. mellifera* was within the range of 118.3 to 124 g/kg DM and *C. alexandrii* of 65.4 to 101 g/kg DM, reported by Marius (2016) and MAWF *et al*. (2017). Proper rumen microbial functioning requires CP ≥ 8% (McDonald, 2011), hence it would appear both encroacher species provide adequate levels of CP for maintenance, but not for productive functions. During droughts and growing seasons, most trees and shrubs remain green, with the leaves which have high amount of crude protein (Topps, 1992). Osuga *et al*. (2008) reported that leguminous tree species like *S. mellifera* have moderate concentrations of crude protein (CP ≥ 8%) and can be essential for proper rumen microbial functioning, particularly during the dry season when only low quality forages are available. The bushes and shrubs can thus be used by farmers as fodder under desert conditions mainly for maintenance and sometimes for production (van Niekert *et al*., 2004).

Neutral Detergent Fibre (NDF) is the primary chemical component of feeds that determines feed digestibility (McDonald *et al*., 2011), with feed digestibility being inversely proportional to the NDF concentration. NDF of both shrubs was higher than the recommended concentrations of 550g/kg DM (Van Soest, 1965), implying the feeds would be slowly digested hence reducing intake. The high content in NDF, ADF and ADL in plant species is associated with increasing maturity. The recommended amount of NDF in animal nutrition should range between 470 to 530 g/kg DM (Buthelezi *et al.*, 2018). High amount of NDF reduces the degradability of nutrients in the feed. The values for fiber fractions of *S. mellifera* and *C. alexandrii* milled twigs with leaves in this study were higher than the cited standards. The *S. mellifera* and *C. alexandrii* samples were obtained in April which is the end of the rainy season when some shrubs were starting to shed their leaves, which may explain the high fibre fraction (NDF, ADF) concentrations as a result of increasing maturity and reduced ratio of leaves to twigs. The tissue properties of stems change greatly with maturity (Cherney and Marten, 1982): mature stems have lower digestibility than leaves due to lignification of stem tissues such as the parenchyma cells which constitutes a strong barrier to digestion of fiber (Wilson, 1993).

The leaves of leguminous bush species which includes *S. mellifera, A. tortilis, and D. cenerea* are associated with low NDF content (20-35%) and usually have a greater digestibility (Elseed *et al*, 2002; Marius, 2016 and Tesfay *et al*., 2018). Low NDF concentration leads to a greater fermentation rate, hence improving the digestibility rate of the forage (Van Soest, 1994). Calcium (Ca) and phosphorus (P) are some of the essential nutrients that are responsible for bone and teeth formation in livestock and the recommended ratio of Ca:P should be in the range of 1:1 to 2:1, but ruminants apparently can tolerate a much wider margin as long as the phosphorous requirements are met (McDonald *et al*., 2007). The Ca:P ratio in this study was 13.1:1 for *S. mellifera* and 7.3:1 for *C. alexandrii*. Marius (2016) reported Ca:P ratio of 7.3:1 for *S. mellifera* and 8.2:1 for *C. alexandrii* in milled leaves.

Variation in the concentration of minerals in forages is influenced by many factors including climate, soil, stage of maturity and season (Spears, 1994). Both shrubs had a high (> 90 g/kg) DM concentration which was due to a relatively high proportion of twigs compared to the leaves since the harvesting was done late in the growing season when some shrubs were already beginning to shade leaves.

3.6. Conclusion

Senegalia mellifera and *C. alexandrii* being some of the most dominant encroacher species in Namibia have moderate nutritional value with high lignocellulose that constraints the availability of their nutrients, most notably the nitrogen (N) component. Hence feeding of these encroacher bush species without chemical treatments and supplements may not provide sufficient nutrients even for maintenance in livestock. Since fibre content was observed to be high in both encroacher species investigated, the use of chemical treatments and supplements during feed formulation may help in reducing the fibre content and improving the level of crude protein, respectively.

CHAPTER 4

EFFECTS OF SODIUM HDROXIDE AND UREA TREATMENT ON THE CHEMICAL COMPOSITION OF *SENEGALIA MELLIFERA*

4.1. Introduction

Encroacher species contain high amounts of cellulose, hemicellulose and lignin biomass especially when small branches and twigs of these bushes are milled (GIZ *et al*., 2017). Additionally, high amounts of cellulose, hemicellulose and lignin materials tend to decrease metabolisable energy and digestibility because of high indigestible fibre content resulting in inaccessibility of the nutrients to the animals. Therefore, it is important to investigate the effects of chemical treatment on the nutritional value of encroacher bush species. Bush species that are prepared for animal feeds should be treated with chemicals that help in increasing the digestibility and palatability of bush-based animal feeds. Sodium hydroxide and urea have been widely used in improving animal feeds that are of low nutritional values (Mynhardt, 2016).

The use of chemical treatment also improves the voluntary feed intake and degradability of the fibrous materials. Therefore, the objective of this study was to determine the effect of NaOH and urea treatment at different levels (4%, 6% and 8%) on chemical composition of *S. mellifera* milled samples.

4.2. Materials and Methods

Harvested samples of *Senegalia mellifera* from Neudamm campus farm were chopped by using a wood chipper (Weifang Fred Machinery Co, Xiaoxujia Industrial Estate, China) and then milled in a hammer mill with a 4 mm sieve. The application rates of 4, 6 and 8 kg of urea and NaOH per 100 kg of bush mill matter was used during treatment. Milled bush material was then treated with sodium hydroxide (NaOH) and urea at different levels (0%, 4%, 6% and 8%) and each level of treatment was replicated two times. The treated samples (control, urea and NaOH) of milled *Senegalia mellifera* were filled into plastic bags with approximately 0.5 mm thickness, then tied up and kept at room temperature on cemented floor for 21 days in a laboratory. After 21 days of incubation, the materials were removed from the plastics and allowed to dry in order to avoid mold, thereafter they were analysed at MAWF laboratory for chemical composition.

4.3. Laboratory analysis

Samples of differently treated *Senegalia mellifera* bush mill and formulated bush-based feeds were analysed for dry matter (DM), moisture, organic matter (OM), ash content, crude protein (CP), crude fiber (CF), ether extract (EE) and ash using AOAC (1995) procedures. The fibre content which includes neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude fibre (CF) were determined according to (Van Soest *et al*., 1991). Metabolizable energy (ME) was obtained using a prediction equation derived from Menke and Steingass (1988). Calcium and phosphorus were also analyzed following the procedures indicated in chapter 3 (pg 38 section 3.2.2). Hemicelulose concentration was estimated by difference as below:

Hemicellulose (%) = %NDF - %ADF

Where: NDF is the neutral detergent fibre and ADF is the acid detergent fibre which are both expressed on dry matter basis.

4.4 Statistical analysis

Data obtained for the chemical analysis were subjected to the one way analysis of variance (SAS, 2008) following a completely randomized design, but with subsampling. The experiment unit was the bag of milled *S. mellifera* which had been treated in a specific way (0, 4, 6 and 8% NaOH or urea) and from which two subsamples were obtained for laboratory analysis. Due to the subsampling, the correct error term for testing significant effects was therefore the experimental error (Lentner and Bishop, 1993). Phosphorous values were not normally distributed and attempts to transform them to a normal distribution were unsuccessful, hence the Kruskal-Wallis nonparametric test was used. Effects were considered significant at $P < 0.05$. A trend was recognized at $0.05 \leq P \leq 0.1$. The model used for the analysis was:

$$y_{ijk} = \mu + \tau_i + \varepsilon_{ij} + {}^{\delta}ijk$$

Where:

$yijk$ = observation for the k-th subsample on the j-th experimental unit (EU) of the i-th treatment

μ = the overall mean

τ_i = the fixed effect of treatment *i*

ε_{ij} = random component explaining the variation among EU on the same treatment

${}^{\delta}ijk$ = random component explaining variation within EU, among subsamples of the same EU.

4.5. Results

4.5.1 Chemical composition of treated Senegalia mellifera samples

The results of chemical composition of treated *Senegalia mellifera* are presented in Table 4.2. The DM, OM, CP, EE, ADF, NDF, ash content were influenced ($P < 0.0001$) by the treatment. Treatment with NaOH reduced DM compared to other treatments, with most pronounced effects evident for NaOH6 and NaOH8. Organic matter concentration was elevated ($P <.05$) in NaOH6 and NaOH8 compared to other treatments; NaOH6 and NaOH8 did not differ ($P >.05$) in their effect on OM. NDF was lower ($P <.05$) in samples treated with NaOH4 and urea8 than NaOH6 or NaOH8 (Table 4.4). Nonetheless, chemically treated samples did not differ ($P >.05$) in their NDF concentration from controls. Urea8 lowered ($P <.05$) ADF compared to all other treatments. Similarly, urea4 treatment was more effective in lowering ($P <.05$) ADF than CONTRL, urea6 and NaOH8. Treatment with NaOH4, urea4, urea6 and urea8 increased ($P <.05$) CP concentration compared to CONTRL. Treatment with urea8 tended ($P <.100$) to increase CP compared to urea4 and urea6. Ash content was reduced ($P <.05$) for NaOH6 and NaOH8 treatments compared to all other treatments. There was no difference ($p >.05$) in phosphorus concentration of the *S. Mellifera* samples (Table 4.3).

Table 4.2. Chemical composition (g/kg DM) of treated *Senegalia mellifera* leaves and small branches*

*****DM = Dry Matter; OM = Organic Matter; Moisture; CP = Crude Protein; Ca = Calcium; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; EE = Ether Extract; NaOH = Sodium Hydroxide. urea4 = 4% urea; urea6 = 6% urea; urea8 =8% urea; NaOH4 = 4% NaOH; NaOH6 = 6% NaOH; NaOH8 = 8% NaOH; CTRL = control.

Variables	Urea4	Urea6	Urea8	NaOH4	NaOH6	NaOH8	CTRL	R^2	CV	S.E	P-Value
DM	934.0^{bd}	946.6^{b}	943.8^{b}	931.8^{cd}	918.9^{a}	917.4^{a}	943.7^{b}	0.85	0.7	0.3	< .0001
ASH	145.3^{b}	147.1^{b}	153.4	141.7^{b}	88.9^{a}	94.0^{a}	142.4^{b}	0.68	18.6	1.2	< .0001
OM	854.7^{a}	852.9^{a}	846.6^{a}	858.3^{a}	911.1^{b}	905.9^{b}	857.7^{a}	0.68	2.8	1.2	< .0001
CP	117.5^{b}	115.9^{b}	149.1^{b}	121.5^{b}	84.0^{a}	84.1^{a}	80.0^{a}	0.69	20.9	1.2	< .0001
NDF	628.8^{ab}	637.4^{ab}	610.1^{a}	619.2^{a}	$656.2a^{b}$	$653.5a^{b}$	629.3^{a}	0.50	3.5	1.1	< .0001
ADF	506.3^{b}	522.9^{c}	470.8^{a}	513.1^{bc}	519.4^{bc}	527.5^{c}	527.1^{c}	0.86	2.1	0.5	<.0001
EE	11.5^{a}	17.0^{bc}	19.9^{c}	17.2^{bc}	18.6^{c}	12.8^{a}	14.4^{ab}	0.7	16.2	0.1	< .0001
Ca	14.4^{ab}	10.8^{a}	15.4^{b}	16.4^{b}	14.5^{ab}	14.2^{ab}	18.3^{b}	0.7	16.5	0.2	< .0001

Tables 4.4 and 4.5 show planned contrasts for the different treatments. Treatments with NaOH and urea reduced ($P < 0.05$) DM compared to controls. Treatment with NaOH drastically reduced ($P < .0001$) DM compared to urea; treatment with NaOH6 and NaOH8 led to a greater reduction ($P = 0.005$) in DM compared to NaOH4. The planned contrasts in Table 4.4 indicate NaOH treatment increased ($P = 0.001$) OM compared to urea; NaOH6 and NaOH8 treatments increased OM much higher ($P = 0.004$) than NaOH4 (Table 4.5). Treatment with NaOH4 reduced ($P = 0.02$) NDF compared to treatment with NaOH6 and NaOH8. The planned contrasts show that urea treatment tended to be more effective ($P = 0.07$) in reducing NDF than NaOH. Treatment with urea and NaOH increased ($P < .05$) CP compared to the CONTRL. Urea treatment was more effective ($P = 0.005$) than NaOH in increasing CP concentration. Ash concentration was lower ($P = 0.001$) under NaOH6 and NaOH8 treatments compared to urea.

Table 4.3. Wilcoxon score (rank sums) used in Krusk-Wallis non-parametric test for phosphorus (P) concentration (%DM) of treated *Senegalia mellifera* with urea and sodium hydroxide.

Treatment	n	Sum of scores	Mean scores
Urea4	4	68	17
Urea6	4	38	9.5
Urea8	4	64	16
NaOH4	4	61	15.25
NaOH6	4	37	9.25
NaOH8	4	62	15.5
Control	4	76	19

Table 4.4. Planned contrasts of least squares means (g /kg DM) for DM, NDF and ADF*

Contrast	estimate	S.E	P value
DM			
CTRL vs. (urea, NaOH)	6.94	2.12	0.006
NaOH vs. urea	-5.64	0.80	<.0001
NaOH4 vs. (NaOH6, NaOH8)	2.71	0.80	0.005
urea4 vs (urea6,urea8)	-2.24	0.80	0.014
NDF			
CTRL vs. (urea, NaOH)	-2.93	7.19	0.699
NaOH vs. urea	5.26	2.72	0.073
NaOH4 vs. (NaOH6, NaOH8)	-7.13	2.72	0.020
urea4 vs (urea6, urea8)	1.02	2.72	0.714
ADF			
CTRL vs. (urea, NaOH)	10.27	3.42	0.009
NaOH vs. urea	6.003	1.29	0.000
NaOH4 vs. (NaOH6, NaOH8)	-2.08	1.29	0.129
urea4 vs (urea6,urea8)	1.90	1.29	0.163

*urea4 = 4% urea; urea6 = 6% urea; urea8 =8% urea; NaOH4 = 4% NaOH; NaOH6 = 6% NaOH; NaOH8 = 8% NaOH; CTRL = control.

Table 4.5. Planned contrasts of least squares means (g/kg DM) for Ca, fat, OM and ash*

Contrast	estimate	S.E	P-value
Ca			

CTRL vs. (urea, NaOH)	2.36	0.80	0.011
NaOH vs. urea	0.40	0.30	0.203
NaOH4 vs. (NaOH6, NaOH8)	0.41	0.30	0.198
urea4 vs (urea6,urea8)	0.38	0.30	0.232
Fat			
CTRL vs. (urea, NaOH)	-1.06	0.85	0.233
NaOH vs. urea	0.004	0.32	0.991
NaOH4 vs. (NaOH6, NaOH8)	0.30	0.32	0.370
urea4 vs (urea6,urea8)	-1.39	0.32	0.0007
OM			
CTRL vs. (urea, NaOH)	-8.36	7.84	0.305
NaOH vs. urea	12.13	2.96	0.001
NaOH4 vs. (NaOH6, NaOH8)	-10.04	2.96	0.004
urea4 vs (urea6, urea8)	0.99	2.96	0.743
Ash			
CTRL vs. (urea, NaOH)	8.36	7.84	0.305
NaOH vs. urea	-12.13	2.96	0.001
NaOH4 vs. (NaOH6, NaOH8)	10.04	2.96	0.004
urea4 vs (urea6, urea8)	-0.99	2.96	0.743

*urea4 = 4% urea; urea6 = 6% urea; urea8 =8% urea; NaOH4 = 4% NaOH; NaOH6 = 6% NaOH; NaOH8 = 8% NaOH; CTRL = control.

4.6. Discussion

Treatment of *Senegalia mellifera* samples with NaOH and urea reduced ADF concentration which is in agreement with Wanapat *et al*. (2009) who reported a reduction in NDF and ADF concentration when rice straw was treated with 5% urea. Concentrated alkaline agents break the ester bonds between the lignin, hemicelluloses and cellulose; physically they cause swelling of the fibres. As a result, the structural carbohydrates are more easily attacked by rumen microbes, increasing digestibility and palatability of the feed (Wanapat and Cherdthong, 2009). It should be noted that milled *S. mellifera* leaves and small branches concentrations of NDF and ADF were of comparable magnitude to that reported for rice straw (Wanapat *et al*., 2009) and hence it is encouraging that alkali treatment was beneficial in reducing the fibre content.

The results are perplexing in that although the alkaline treatments had no significant effect on NDF concentration, the urea4 and urea8 treatments reduced ($P < 0.05$) ADF. The results also indicate that urea8 and NaOH4 were most effective in reducing ADF concentrations and simultaneously also increased CP concentration. Decreasing the concentration of NDF and ADF in feeds permits easy degradability of nutrients by ruminal microbes such as bacteria, fungi and protozoa (Murta *et al*., 2011). By increasing NDF digestibility for low nutritional feeds, the daily dry matter intake can be enhanced, because there is a negative relationship between NDF and digestibility of feeds (McDonald *et al*. 2002). Although untreated *S. mellifera* samples had a CP of 8 g/kg DM which would meet the minimum threshold for rumen microbial activity, this might not all be available resulting in deficiencies, hence alkali treatment appears to have an added benefit which would elevate CP levels to satisfy proper rumen functioning. Differences observed in the effect of the different alkaline concentrations are because the rate of delignification is affected by the concentration of hydroxide ions and overall pH (Dolk *et al*., 1989). We surmise that just like in the conversion of cellulose into fermentable monomers, during the production of renewable fuels, the effectiveness of the different alkaline pre-treatment options may be influenced by the physical/chemical composition of the biomass and the pre-treatment conditions which include chemical used, chemical concentration, moisture, temperature, pH and length of treatment (Chen *et al*., 2013).

The application of urea treatment can be more effective when the forage has moisture value of about 30 % (Dolberg, 1992). The precise concentration of alkaline agents used to treat bush material, may be influenced by season, species and location from where it was harvested. From a practical perspective it would thus appear that different bush feed recipes may be recommended for farmers from different agroecological zones, depending on the predominant encroacher bush species. It may also be desirable that farmers send their milled bush samples harvested during different seasons, for chemical analyses prior to feed formulation. Urea treatment may be preferable to use of NaOH because the latter is more difficult to handle at farm level due to its caustic nature (Adesogan *et al*., 2019) while urea also enhances the nitrogen content. To be most effective, urea treatment requires adequate urease and moisture (Adesogan *et al*., 2019). Other alternatives, however, need to be explored for efficacy of treating bush material and their price competitiveness. For example, ammonia fibre expansion (AFEX) which entails exposure of straw to high levels of ammonia at elevated temperatures and pressure for less than 1 hour, increased the digestibility and energy availability of wheat straw for lactating buffalo and cattle (Mor *et al*., 2018). Similarly, a combination of urea and lime to treat rice straws improved the degradability and also increased the crude protein than using lime or urea alone (Wanapat *et al*., 2013). The results in this study need further confirmation before they can be of practical use by farmers. It would appear that further optimization of the treatment conditions of milled bush feed material is required including chemicals used (NaOH, CaOH, CaO, ammonia, urea), chemical concentration, pH, moisture, temperature and duration.

4.7. Conclusion

Treatment of milled *S. mellifera* bush feed material using NaOH4 and urea8 was effective in improving its nutritive feeding value for ruminants, and may be useful particularly when it is used in formulating diets. More research is needed to establish optimal concentrations and conditions (pH, moisture content, temperature, duration) for effective treatment of *S. mellifera* bush feed material so it can be used in feeding. This is because although ADF concentration was significantly reduced with NaOH4 and urea8 treatments, the effects on NDF were less convincing.

CHAPTER 5

PALATABILITY OF THE FEEDS DEVELOPED FROM *SENEGALIA MELLIFERA* ENCROACHER BUSH WHEN FED TO WEANED DAMARA SHEEP

5.1. Introduction

Feeds produced from *Senegalia mellifera* have not been considered as an alternative feed to livestock during the dry season when rangelands cannot provide quality grazing resources. Encroacher bush could be used to feed animals during periods of feed scarcity. Despite the ready availability of encroacher bush species such as *Senegalia mellifera,* use of bush-based animal feeds is limited by poor voluntary intake and low crude protein concentration, which is associated with poor degradability (GIZ, 2016). Utilization of poor quality forages for example wheat straw for feeding livestock may be constrained by palatability (Ng'ambi and Ngosa, 1995). Alkali treatment (e.g. NaOH and urea) of crop residues improved feeding value in ruminants (Ali and Jabbar, 2014). Sundstol *et al.* (1978) recommended the use of urea and ammonia in improving digestibility and intake.

Supplementing ruminant diets with exogenous fibrolytic enzymes has helped improve fibre digestibility, resulting in improved efficiency of feed utilization as reviewed by Sujani and Seresinhe (2015), although some studies did not report any significant performance improvements. The objectives of this experiment were to determine: (i) the nutrient composition of formulated diets from chemically and fibrolytic enzyme treated *Senegalia mellifera* samples; and (ii) the palatability of the feeds developed from *Senegalia mellifera,* when fed to weaned Damara sheep.

5.2. Materials and Methods

Based on the results of the chemical treatment of the *S. mellifera* samples in Chapter 4 which indicated a significant reduction in ADF at 4% concentration for both NaOH and urea treatments compared to controls, 2 of the diets were formulated using this treatment level; the other diets were the control and fibrolytic enzymes. The four diets (control, Urea4D, NaOH4D and Enzyme4D) were formulated to be isonitrogenous as shown on Table 5.1 below, with a composition of 40% bush mill, 10% *Vachellia erioloba* pods, 25% marula oil cake, 15% yellow maize, 5% molasses and 1% minerals. The same protocols were as in Chapter 4 were used in chemical analysis of two subsamples of each formulated feed. The fibrolytic enzyme (xylanase and cellulase) was applied at 4% concentration (enzyme4) on the day the samples were analysed in the laboratory for chemical composition.

For the palatability experiment, guidelines of the University of Namibia on the welfare of animals were followed. A Randomized Complete Block Design with sex as the blocking factor, was used for the palatability trial with four diets (control, 4% urea, 4% NaOH and fibrolytic enzymes) randomly allocated to growing sheep (Appendix 8.12, Figure 5.1). Sixteen male and female weaned Damara sheep of four to five months of age were confined in individual pens of 2.5m x 3m. The trial consisted of a 5-day adaptation period, followed by a 12-day trial period. Sheep were weighed at the beginning and end of the adaptation period and also at the end of the feeding trial period.

The Damara sheep were treated for internal and external parasites before the beginning of the trial using IVOMEC ® (Merial, South Africa). The adaptation period was necessary for familiarizing sheep to the bush-based diets and confinement. Each pen had a water container and a feed trough. Pens were situated in a roofed house with open sides. Diets were formulated based on treated *S. mellifera* and other ingredients as in Table 4.1. The feeds were offered at 09h00 and 15h00 with allowance of six hours feeding time. The refusals were collected, weighed and intake determined by difference. The pens were cleaned and residues of the previous day's feeds were removed before fresh feed was provided.

Table 5.1. The feed ingredients and nutritional composition of different bush feed rations

	Diets			
Ingredients (g/kg)	**0 (control)**	**Urea4D**	**NaOH4D**	**Enzymes4D**
Bush mill	400	400	400	400
Yellow maize	150	150	150	150
Marula oil cake	250	250	250	250
Camel thorn pods	100	100	100	100
Molasses	50	50	50	50
Coarse salt	50	10	10	10
NaOH	0	0	40	0
Urea	0	40	0	0
Enzymes	0	0	0	40
Total	**1000**	**1000**	**1000**	**1000**
Nutrient composition of diets				
CP (g/kg DM)	101.8	125.3	110.4	112.7
NDF (g/kg DM)	481	405.1	350.3	460.2

ME (MJ/kg)	13.5	11.2	11.5	10.8
EE (g/kg DM)	70.9	95.9	98.4	60.5
Ca (g/kg DM)	8.5	6.4	6.7	8.3
Target CP (g/kg)	**10%**	**10%**	**10%**	**10%**

5.3. Statistical analysis

Data obtained for the chemical analysis were subjected to the one way analysis of variance (SAS, 2008) following a completely randomized design, but with subsampling. The experiment unit was the bag of milled *S. mellifera* which had been formulated in a specific way (control, NaOH4D, urea4D and enzyme4D) and from which two subsamples were obtained for laboratory analysis. Due to the subsampling, the correct error term for testing significant effects was therefore the experimental error (Lentner and Bishop, 1993). Phosphorous values were not normally distributed and attempts to transform them to a normal distribution were unsuccessful, hence the Kruskal-Wallis nonparametric test was used. Effects were considered significant at $P < 0.05$. A trend was recognized at $0.05 \leq P \leq 0.1$.

Feed intake was analyzed as a randomized complete block design with repeated measurements using the Proc Mixed procedure (SAS, 2008). The Bayesian Information Criteria (BIC) was used to select the best covariance structure, which was used in modeling (Litell *et al*, 1998). The Heterogeneous autoregressive covariance structure was selected as best. Effects were considered significant at $P < 0.05$, post hoc analyses were carried out in order to check the difference in means. A trend was recognized at $0.05 \leq P \leq 0.1$.

The model for analysis was:

$$y_{ijk} = \mu + \tau_i + t_j + (\tau * t)_{ij} + \varepsilon_{ijk}$$

Where:

y_{ijk} = observation on experimental unit k, on day j, given diet i.

μ = the overall mean

τ_i = the effect of treatment i

t_j = the effect of day j

$(\tau * t)_{ij}$ = the effect of interaction between treatment i and day j

ε_{ijk} = random error

5.4. Results

5.4.1. Chemical composition of formulated diets from *Senegalia mellifera*

Formulated diets differed (P <.05) in their DM, OM, EE, NDF, ADF and ash (Table 5.2). Enzyme treated diets, however, did not differ (P >.05) in their NDF concentration from the control. The control diet had higher (P <.05) NDF concentration compared to urea4D and NaOH4D. The control diet did not differ (P >.05) from enzyme4D in the Ca concentration. Chemically treated diets (urea4D, NaOH4D) had a lower (P <.05) Ca concentration than the control. On the other hand, chemically treated diets (urea4D, NaOH4D) had elevated levels of phosphorous compared to the control and enzyme4D diets. Similarly, chemically treated diets (urea4D, NaOH4D) had higher (P <.05) concentration of EE compared to control and enzyme4D diets. The four diets did not differ (P >.05) in CP concentration because they were formulated to be iso-nitrogenous.

Table 5.2. Nutritional composition of diets formulated from *Senegalia mellifera**

Variables (g/kg DM)	Control	Urea4D	NaOH4D	Enzyme4D	R^2	C.V	S.E	P-values
DM	924.9^{b}	937.8^{d}	916.7^{a}	931.2^{c}	0.9	0.5	0.2	< .0001
Ash	86.7^{a}	91.9^{a}	84.1^{a}	88.6^{a}	0.2	9.8	0.4	< .0001
OM	913.3^{a}	908.03^{a}	915.9^{a}	911.4^{a}	0.2	0.9	0.4	< .0001
CP	99.6^{a}	109.3^{b}	105.8^{ab}	101.1^{ab}	0.7	5.04	0.2	< .0001
NDF	481.01^{c}	405.2^{b}	350.3^{a}	460.2^{c}	0.9	6.01	1.2	< .0001
ADF	325.3^{c}	284.2^{b}	234.9^{a}	289.2^{c}	0.9	5.02	0.7	< .0001
Hemicellulose	155.7^{b}	120.9^{a}	115.4^{a}	170.7^{b}	0.9	5.9	0.6	< .0001
EE	70.9^{b}	95.9^{c}	98.4^{c}	60.5^{a}	0.9	5.8	0.2	< .0001
ME (MJ/kg)	13.5^{a}	11.2^{ab}	11.5^{c}	10.8^{c}	0.7	4.8	0.2	< .0001
Phosphorous	2.6^{a}	3.2^{b}	3.6^{c}	2.4^{a}	0.83	5.4	0.05	< .0001
Calcium	8.46^{b}	6.4^{a}	6.7^{a}	8.3^{b}	0.9	7.7	0.03	< .0001

*Variables with different superscript across the rows are different (p <.05). DM = Dry Matter; OM = Organic Matter; Moisture; CP = Crude Protein; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; EE = Ether Extract; ME = Metabolisable Energy.

urea4D = diet formulated with *S. mellifera* treated with 4% urea; NaOH4D = diet formulated with *S. mellifera* treated with 4% NaOH; enzyme4D = diet formulated with *S. mellifera* treated with fibrolytic enzyme at 4%.

5.4.2. Palatability results

Feed intake was influenced (P <.05) by day and diet x day interaction. Least squares means of daily intake of different *S. mellifera* bush-based diets fed to the weaned Damara sheep are given in Figure 5.1. The widest fluctuations in feed intake in the first 8 days of the trial were in the control and enzyme4D diets. From day 8 to 12, the feed intake appears to have stabilized across all the diets. All the sheep showed some weight increment at the end of the trial period, but the weight data was not analysed because of the short duration of the feeding period.

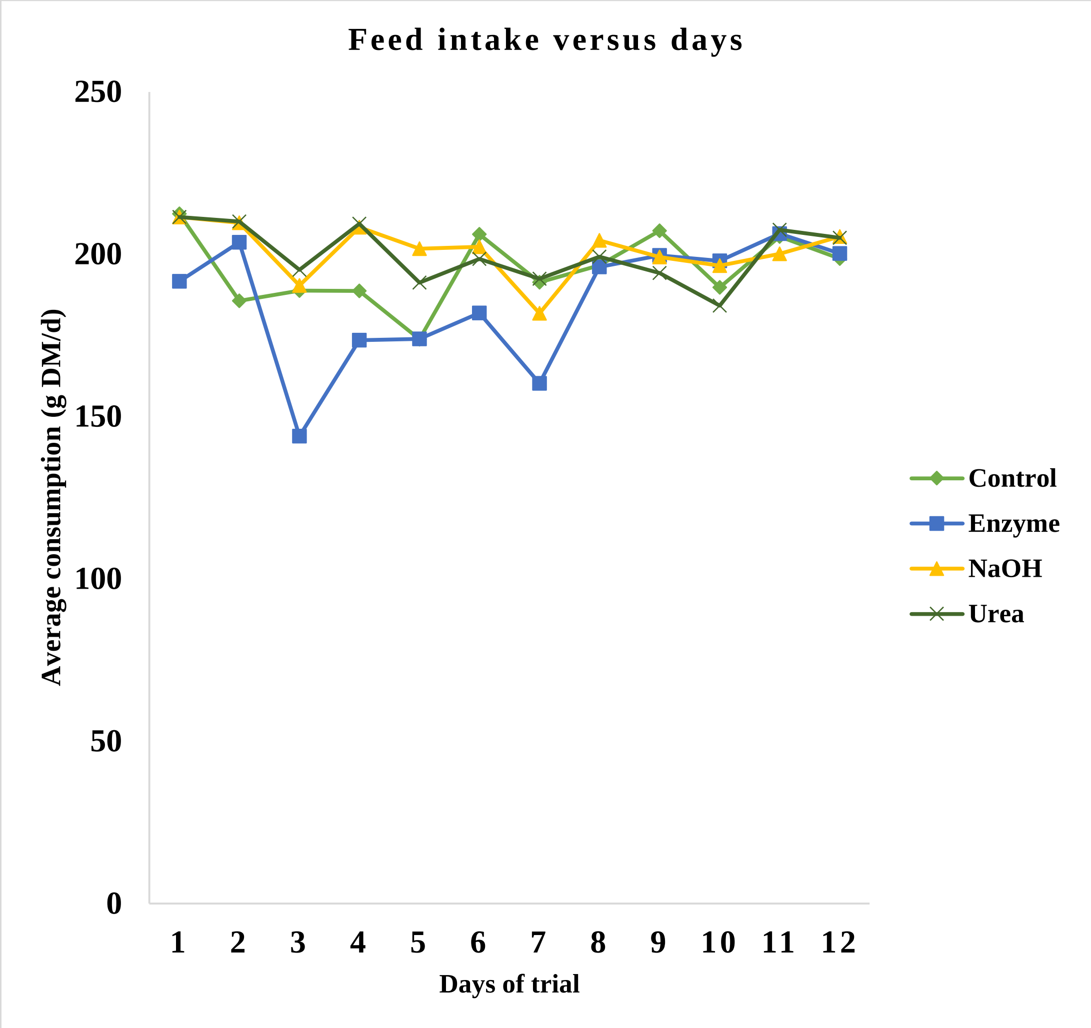

Figure 5.1. Least squares means of feed intake of different *S. mellifera* based diets (control, NaOH4D, urea4D and enzyme4D) fed to weaned Damara sheep.

5.5. Discussion

Diets formulated from chemically treated *S. mellifera* were lower in NDF and ADF concentrations, which may suggest improved nutritive value because of the breakage of ester bonds between the cellulose, hemicelluloses and the lignin. This would present opportunities in utilizing encroacher bush, because bush-based feed in Namibia is presently based on untreated bush material (GIZ, 2017). The mixing of treated milled bush material with other ingredients improves the nutritive of the diet by providing readily fermentable sugars (molasses), rumen degradable protein and minerals which may support increased rumen microbial activity for utilization of bush feed. The enzyme treatment did not affect chemical composition of formulated diets from *S. mellifera*, which is not surprising because the enzymatic activity requires an appropriate environment which is only available once the feed has been ingested and reached the rumen.

The two diets (NaOH4D and urea4D) were selected for the palatability trial based on a reduction in ADF; the third diet was the enzyme4D, because of the improved fibre digestibility attributed to fibrolytic enzymes (Sujani and Seresinhe, 2015); the fourth diet was the control. All the diets were iso-nitrogenous with a CP of 10% which was in excess of the 8% required for rumen microbial activity (McDonald *et al*., 2007) and in addition contained molasses which provided readily fermentable sugars and also enhanced palatability. It has been reported that goats consumed 37% more wheat straw organic matter after it had been sprayed by molasses to improve taste and smell (Ng'ambi and Ngosa, 1995), which supports the ready consumption of the *S. mellifera*-based diets observed with the Damara sheep in our study

The NDF concentration of the four diets was also within the acceptable range (< 550 g DM/kg) for ruminant feeding (McDonald *et al*., 2011). Hence the diet formulations, appeared to have been satisfactory for maintenance requirements of the Damara sheep (given that none of the experimental sheep lost weight and in fact gained weight), although fluctuations in intake were still evident after the 5-day adjustment period. The fluctuations during the trial period would suggest that sheep fed bush-based feed may require a longer adjustment period of more than the 5 – 10 day period recommended in some studies using other ingredients (Kaitho *et al*., 1996). Based on our study, the adjustment period for Damara sheep given *S. mellifera* bush-based feed is at least 12 days.

Growing male goats fed wheat straw treated with urea, enzyme and molasses significantly increased in live weight (Allam *et al.*, 2009). All the experimental animals used in our study maintained or gained weight, showing the potential of *S. mellifera* bush-based feed, however, the data was not analysed because of the short duration of the feeding period. Also of importance to note was that none of the Damara sheep showed any signs of ill health during the trial period. Animal and plant factors are some of the few factors that might have influenced the differences in dry matter intake as observed in this study. In addition, the presence of tannins which are anti-nutritional factors might also have affected the palatability of bush-based feeds. The control and enzyme4D treated diets were similar ($P > 0.05$) in their NDF concentration, which was higher than for the NaOH4D and urea4D diets and this may have adversely affected intake.

Voluntary intake is affected by rumen fill and diets with high NDF concentration will have a reduced passage rate and hence reduced feed intake (Allen, 1996). Although Neutral detergent fiber (NDF) has been used as the only feed aspect to predict the fill effects of forage (Merten, 1987), it may be unsatisfactory and other attributes may influence its fill effects including: initial particle size, fragility of particles, rate and extent of NDF digestion (Allen, 1996). We surmise that the gradual stabilization in the feed intakes of the different diets, may be due to changes in the rumen microbial populations with the bush-based feed material, but this requires further investigation.

5.6. Conclusion

Diets formulated from *S. mellifera* treated with CONTRL, NaOH4, urea4 and enzyme4 were all palatable, although CONTRL and enzyme4 required a longer adjustment period, which may be due to the higher NDF concentration. Treatment of *S. mellifera* bush-based feed with NaOH4 and urea4 improves intake and this is enhanced by inclusion of molasses and other ingredients (marula oil cake, yellow maize, minerals) in the formulated diets, hence enhancing the utility of this resource for feeding ruminants.

CHAPTER 6

CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS

6.1. Conclusions

- Differences ($P < .05$) in chemical composition of *Senegalia mellifera* and *Catophractes alexandrii* were observed for DM, ash OM, CP, NDF, ADF, Ca, and Phosphorous concentration. Both bush species had unacceptably high NDF (> 550 g/kg DM) concentrations which would contribute to rumen fill, hence reducing intake. *Senegalia mellifera* had a higher CP concentration compared to *C. alexandrii*, which would make it preferable for feeding livestock.
- Treatment of *S. mellifera* with NaOH at concentrations of 4%, reduced NDF concentration. Urea at a concentration of 8% reduced ($P < .05$) the ADF concentration compared to all other treatments. Urea and NaOH at a concentration of 4% lowered ($P < .05$) ADF compared to the control. Crude protein concentration was increased ($P < .05$) by urea treatment of milled *S. mellifera* bush. It would appear that urea and NaOH treatment improve the utility of milled bush through delignification, but the reaction conditions need to be optimized.
- Isonitrogenous diets of 100 g/kg CP on DM basis from milled *S. mellifera* that had been treated with urea and NaOH at a concentration of 4% and used as a basal feed with supplements of yellow maize, marula oil cake, camel thorn pods, molasses and minerals were palatable to weaned Damara sheep. The diet formulated from *S. mellifera* treated with 4% urea had the highest N content (109.3 g/kg DM) than the expected N content of 100 g/kg DM. Feed from milled *S. mellifera* supplemented with the same ingredients above was also palatable, but required a longer adjustment period. Similarly, fibrolytic enzyme

treatment of milled *S. mellifera* at feeding required a longer adjustment period, compared to diets formulated using *S. mellifera* that had been chemically treated with urea or NaOH at 4% concentration. Bush feed nutritional composition is improved by the addition of readily fermentable energy sources (yellow maize and molasses) and rumen degradable protein (marula oil cake, camel thorn pods); the molasses in addition improves the palatability.

- Damara sheep fed diets with a basal diet of milled *S. mellifera* supplemented with energy sources (yellow maize, molasses), rumen degradable protein (marula oil cake, camel thorn pods) and mineral mix require an adjustment period of at least 12 days.

6.2. Recommendations

1. Harvesting specifications for encroacher bush. We speculate that encroacher bush species such as *Senegalia mellifera* should be harvested preferably early during the rainy season when they have high rates of foliage growth and the twig size should be < 2cm, which will minimize the fibre content. Harvesting late in the growing season reduces the ratio of leaves to twigs, which may compromise its nutritive value for ruminants. Twigs and small branches >2cm have high amount of fibre content which decreases the voluntary feed intake.

2. Optimization of treatment conditions. Neither urea nor NaOH at the concentrations of 4, 6 and 8% reduced ($P \geq 0.05$) the NDF which is the primary determinant of fill effects and hence passage rate and voluntary intake (Mertens, 1987). The reaction conditions for treatment of milled *S. mellifera* including chemicals, concentration, moisture content, pH and duration need to be optimized, to allow greater reduction of the lignin concentration and hence utility of encroacher bush-derived feeds.

3. Length of adjustment period. A longer adjustment period of at least 12 days may be required for Damara sheep feeding on diets formulated with *S. mellifera* constituting the basal diet. The basal feed should not exceed 40% in a total mixed ration fed to Damara sheep. Inclusion of molasses during diet formulation improves palatability.

4. Growth trial. This trial only considered palatability of the differing diets formulated with *S. mellifera* as the basal feed. A growth trial is required to assess the growth rates on different diet formulations because bush feed may besides being used for survival feeding, be potentially used in production rations. It is also important to assess the degradation characteristics of bush-based feed using *in vitro* and *in vivo* methods.

Carcasses from slaughtered animals should also be assessed for the effect of bush-based feed on meat quality aspects such as fatty acid profiles.

4. Alternative supplements. Various crop and agro-industrial byproducts may be available in different regions and at different times of the year, hence it is important to test their utility in formulating diets based on bush feed as the basal diet. Pelleting could be investigated for different diets, as a means of enabling distribution of bush-based feed.

5. Cost-benefit analysis of bush-based feed. Production of any feed entails costs and hence it is important to do cost-benefit analyses and compare with alternatives. Bush thinning to reduce encroacher bushes increases the carrying capacity in the medium term and this added benefit should be included in the cost-benefit analyses.

6.3. Limitations of the study

1. Late harvesting of encroacher bush. This led to leaf loss hence negatively affecting nutritive value. Additionally the encroacher bush species found in most agro-ecological zones of Namibia are thorny which makes the harvesting and handling process cumbersome.
2. Recommend: Harvest early during the growing season and process bush feed for immediate use or conserve as silage; additionally bush harvesting should be mechanized, but this requires cost-benefit analyse.
3. Digestibility not assessed for bush feed. Digestibility was not assessed due to unavailability of fistulated animals; also equipment (Daisy incubator) and chemicals were not available for conducting in vitro analysis.
4. Recommend: Future studies should conduct *in vivo* and *in vitro* analyses for NDF and CP digestibility for *S. mellifera* derived feed.
5. Palatability experiment. The study used dried bush feed materials for the palatability trial; results may differ if bush feed is formulated from freshly harvested materials which may have a higher concentration of volatile compounds that are lost upon drying.
6. Changes in chemical composition by season. The quality and quantity of bush-feed materials may vary by season, hence ration formulation may need to take into account these fluctuations and also availability of additional required ingredients (e.g. molasses, Marula oil cake, cotton seed cake).
7. Size specifications may be unrealistic for real-life applications. The harvested materials were up to a size specification of not more than 2cm for the branches, which was possible for small scale laboratory trials but may be unrealistic for industrial-scale operations. The implication is that the chemical composition

of formulated feed and the palatability may have been exaggerated and more conservative values should be used in actual formulations for real field applications.

8. Most of the refusal feeds that remained in the feeding trough were wooden components, this is an indication that animals were selecting the most palatable components of the diet, which may reflect size specifications were not strictly met.
9. Delayed laboratory analyses. The chemical analyses could not all be done on time due to unavailability of required chemicals. Also the CP could not be analyzed at the MAWF laboratory due to a breakdown of the equipment. The Department of Animal Science Nutrition laboratory should be upgraded so that it has the capacity to do most of the chemical composition analyses, so as to improve the quality of training and also service provision to the livestock industry.

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7. Appendices

Appendix 8.1. Sample SAS programm used for analyses of bush feed chemical composition.

```
/* 2-11-2019
Analysis of encroacher bush data for Mr. Hostics Ndozi.

(1) All values for proximate parameters are expressed on DM
basis.
(3) The data set used is:
c:\students\ndozi\data\data\chemical com,position of senegalia
mellifera-based formulated diets 29102019v2.xls
[Cruzer glide USB].


*/

options ps=50 ls=78 nocenter nodate;
title "Chemical analyses of Senegalia mellifera based dites ";

data res1;
input trt $ labno rep  trt_code dm om ash moist cp  ndf adf cf
fat ca phos @@;
cards;
enzyme      240   1     1     93.31765299 91.78461926 8.215380738
      6.682347012 9.89094743  43.58631964 29.27524407 43.4906784
      5.657905072 0.789775542 0.231467459
enzyme      240   2     1     93.20367133 92.00174825 7.998251748
      6.796328671 10.63262837 43.85085272 28.65958289 43.85085272
      5.726323782 0.865845721 0.227458851
enzyme      241   1     1     93.16178916 90.36415032 9.635849684
      6.838210841 9.937569234 48.68524612 29.17758794 48.68524612
      6.33355155  0.808949121 0.250099181
enzyme      241   2     1     92.79158369 90.41042515 9.589574855
      7.208416313 9.965664895 47.95643915 28.577917   47.95643915
      6.480652909 0.846141359 0.24333477
urea  242   1     2     94.04251846 91.77785939 8.222140613
      5.957481536 10.15495465 42.18706423 30.09129733 42.18706423
      9.356047234 0.613437711 0.318159523
urea  242   2     2     94.07081221 91.74450672 8.255493284
      5.929187795 11.42302725 42.45771202 29.78708691 42.45771202
      9.835415057 0.530821972 0.251786192
urea  243   1     2     93.46322295 89.8162171  10.1837829
      6.536777046 10.64395753 38.88908694 26.4187328  38.88908694
      9.576534451 0.686958347 0.370757098
urea  243   2     2     93.5605894  89.87464262 10.12535738
      6.4394106   11.51604023 38.49980018 27.37472556 38.49980018
      9.578834175 0.714328292 0.357164146
naoh  244   1     3     91.76374412 91.62068261 8.379317392
      8.236255876 9.943169752 33.89535528 23.90281281 33.89535528
      10.4048239  0.68652621  0.378735445
naoh  244   2     3     91.52664325 91.53087569 8.469124307
      8.473356753 10.21513225 33.74526656 22.87650018 33.74526656
      10.15008612 0.627110793 0.350701332
```

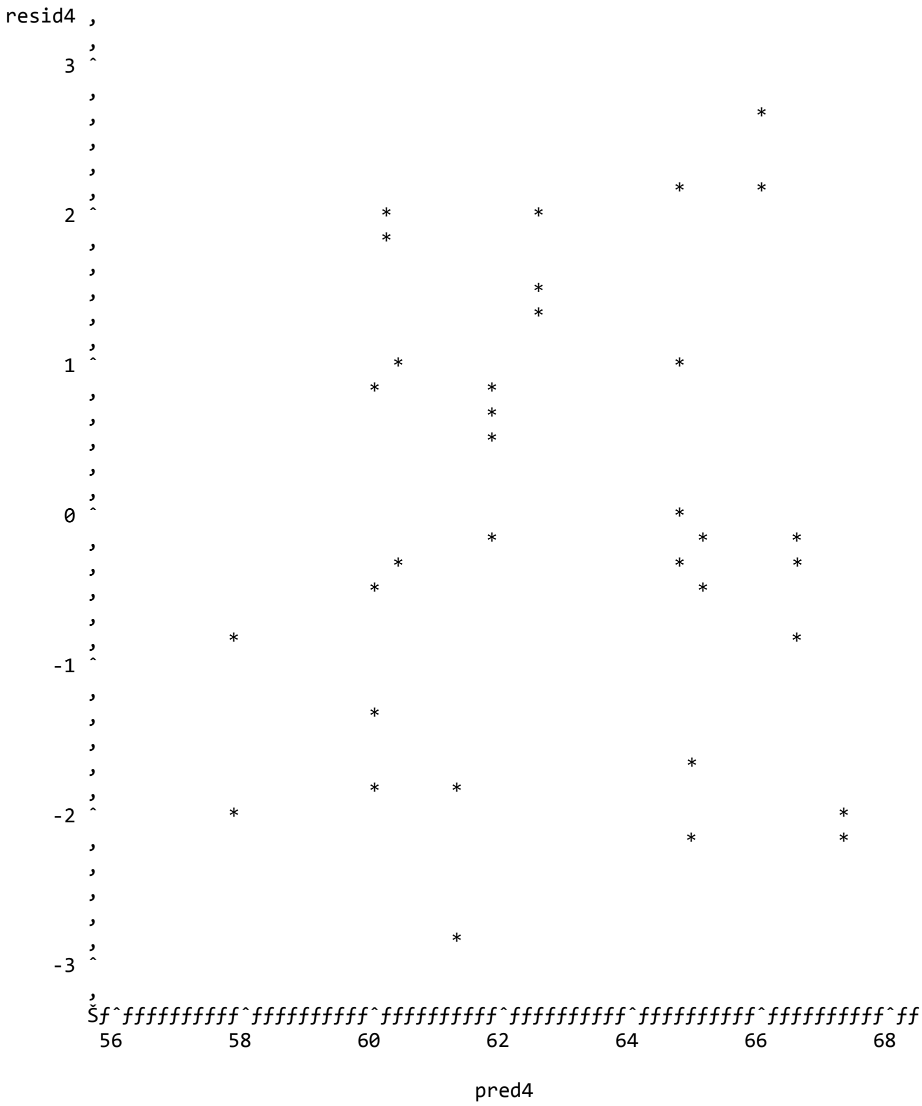
```
naoh  245   1     3     91.32777828 91.78969296 8.210307037
      8.672221719 11.38472051 35.56090527 23.40987372 35.56090527
      9.453349318 0.684172677 0.372590853
naoh  245   2     3     92.04884668 91.40660335 8.593396653
      7.951153324 10.77602672 36.90803869 23.75963694 36.90803869
      9.344497943 0.676099951 0.34352004
control     246   1     4     92.61681439 91.3819809  8.618019103
      7.383185612 10.17706107 49.47737183 33.5435008  54.37098513
      6.642447385 0.861220114 0.25497368
control     246   2     4     92.95067656 91.29637713 8.703622872
      7.04932344  10.25232358 50.02526516 33.96256429 54.88131755
      6.889649072 0.77221027  0.232210748
control     247   1     4     92.78929766 91.37123746 8.628762542
      7.210702341 9.040043924 46.38366892 30.96618661 61.6874012
      7.420278184 0.873360176 0.271420205
control     247   2     4     91.62510202 91.28049333 8.719506665
      8.374897978 10.38557051 46.51926523 31.65187935 62.03571426
      7.416699618 0.876419453 0.273881079
;


data res2a;
set res1;

proc freq;
tables trt/
norow nocol nopercent ;
run;


/* CV =27% which is high! */

data res2c;
set res2a;

label cp='Distribution of cp ';
proc univariate normal def=5; var cp;
proc chart; hbar cp/type=pct midpoints=6.5 to 16 by 0.95;
run;


/* Report results for untransformed NDF */

data res5;
set res2a;

proc glm;
class trt labno rep;
   model ndf=trt rep(trt)/ss3;
   output out=res4 r=resid4 p=pred4;
   random rep(trt);
   test h=trt e=rep(trt);
   lsmeans trt/stderr pdiff;

   estimate 'control vs (enzyme,urea, NaOH)' trt 3 -1 -1 -1;
   estimate 'control vs enzyme' trt 1 -1 0 0;
   estimate 'control vs urea' trt 1 0 0 -1;
   estimate 'control vs NaOH' trt 1 0 -1 0;
```

```
   estimate 'enzyme vs (urea, NaOH)' trt 0 2 -1 -1;

run;

/* Residuals indicate a constant variance. */

data res6;
set res4;
proc plot; plot resid4*pred4='*';
run;



/* Report these results for ADF */

data res7;
set res2a;
proc glm;
class trt labno rep;
   model adf=trt rep(trt)/ss3;
   output out=res8 r=resid5 p=pred5;
   random rep(trt);
   test h=trt e=rep(trt);
   lsmeans trt/stderr pdiff;

   estimate 'control vs (enzyme,urea, NaOH)' trt 3 -1 -1 -1;
   estimate 'control vs enzyme' trt 1 -1 0 0;
   estimate 'control vs urea' trt 1 0 0 -1;
   estimate 'control vs NaOH' trt 1 0 -1 0;
   estimate 'enzyme vs (urea, NaOH)' trt 0 2 -1 -1;

run;

/* Residuals indicate a constant variance. */

data res9;
set res8;
proc plot; plot resid5*pred5='*';
run;



/* Data tranformation for CF using 1/sqrt, improved
residuals to be almost randomly distributed. Use this transformed
data
for reporting in thesis. */
data res33;
set res2a;

*cf2=sqrt(cf);
cf2=1/sqrt(cf);
*cf2=log(cf);

proc glm;
class trt rep;
   model cf2=trt rep(trt)/ss3;
```

```
   random rep(trt);
   output out=res34 r=resid14 p=pred14;
   test h=trt e=rep(trt);
   lsmeans trt/stderr pdiff;

   estimate 'control vs (enzyme,urea, NaOH)' trt 3 -1 -1 -1;
   estimate 'control vs enzyme' trt 1 -1 0 0;
   estimate 'control vs urea' trt 1 0 0 -1;
   estimate 'control vs NaOH' trt 1 0 -1 0;
   estimate 'enzyme vs (urea, NaOH)' trt 0 2 -1 -1;
run;

/* Distribution of residuals is nearly nonrandom.
Attempt data transformation */
data res35;
set res34;
proc plot; plot resid14*pred14='*';
run;

/* Use results for CP below */

data res40;
set res2a;

proc glm;
class trt rep;
   model cp=trt rep(trt)/ss3;
   output out=res41 r=resid16 p=pred16;
   random rep(trt);
   test h=trt e=rep(trt);
   lsmeans trt/stderr pdiff;
   estimate 'control vs (enzyme,urea, NaOH)' trt 3 -1 -1 -1;
   estimate 'control vs enzyme' trt 1 -1 0 0;
   estimate 'control vs urea' trt 1 0 0 -1;
   estimate 'control vs NaOH' trt 1 0 -1 0;
   estimate 'enzyme vs (urea, NaOH)' trt 0 2 -1 -1;

run;

/* Distribution of residuals is random. */
data res42;
set res41;
proc plot; plot resid16*pred16='*';
run;

/* 30-1-2019. Nonparametric analysis for phos */

data res39;
set res2a;

proc npar1way wilcoxon correct=no data=res39;

class trt;
var phos;
*exact wilcoxon;
run;
```

Appendix 8.2. Residual plot **NDF for milled bush feed samples**

```
Plot of resid4*pred4.  Symbol used is '*'.
```




```
NOTE: 2 obs hidden.
```

Appendix 8.3. Residual plot for **Ca milled bush feed samples**

```
                  Plot of resid6*pred6.  Symbol used is '*'.

resid6 ,
       ,
   0.4 ˆ
       ,
       ,                                                          *
       ,
   0.3 ˆ *
       ,
       ,
       ,
   0.2 ˆ
       ,
       ,                                                  *
       ,                     *
   0.1 ˆ
       ,                                                * **
       ,                                                ***  *
       ,                     *
   0.0 ˆ                                                     *
       ,                    *  *                           *
       ,                  ***                             **
       ,                  * *       *                      *
  -0.1 ˆ                            *
       ,
       ,
       ,                                                          *
  -0.2 ˆ
       ,
       ,
       ,                               *
  -0.3 ˆ                               *
       ,
       ,
       ,
  -0.4 ˆ
       ,
       Šƒˆƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒˆƒƒ
        0.4        0.6        0.8        1.0        1.2        1.4        1.6

                                        pred6

NOTE: 7 obs hidden
```

Appendix 8.4. Residual plot for **ADF milled bush feed samples**

```
                  Plot of resid5*pred5.  Symbol used is '*'.

resid5 ,
       ,
     3 ˆ
       ,
       ,
       ,                            *
       ,
       ,
     2 ˆ
       ,       *
       ,                            *  *
       ,
       ,
       ,                             *
     1 ˆ                            *               *
       ,       *                 *  *
       ,                             *       *  *
       ,                             *
       ,                               *
       ,
     0 ˆ                                        *       *          *
       ,                                            *
       ,
       ,                             *
       ,         *        *
       ,          *                                                *
    -1 ˆ         *  *
       ,
       ,                          *
       ,                                               *
       ,                         *
       ,
    -2 ˆ
       ,
       ,
       ,
       ,                                               *
       ,
    -3 ˆ
       ,
       Šƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒ
          47           48           49           50           51           52

                                        pred5

NOTE: 6 obs hidden.
```

Appendix 8.5. Residual plot for **fat milled bush feed samples**

```
                 Plot of resid10*pred10.  Symbol used is '*'.

    resid10 ,
            ,
       0.15 ˆ
            ,
            ,
            ,
            ,               *                  *  *
            ,                               *
       0.10 ˆ
            ,               *                     *
            ,       *                            *
            ,    *
            ,
            ,    *
       0.05 ˆ
            ,                                  *
            ,                     *           *  *
            ,                               *  *
            ,
            ,                   *
       0.00 ˆ                                 **
            ,                    *
            ,
            ,
            ,                    * *
            ,       *           *
      -0.05 ˆ                                               *
            ,                  *
            ,                 **  *
            ,
            ,                      *
            ,                            *                  *
      -0.10 ˆ                       *
            ,
            ,                       *    *
            ,
            ,
            ,
      -0.15 ˆ
            ,
            Šffˆfffffffffffffˆfffffffffffffˆfffffffffffffˆfffffffffffffˆff
              1.0           1.2           1.4           1.6           1.8

                                        pred10

NOTE: 1 obs hidden.
```

Appendix 8.6. Residual plot for **OM milled bush feed samples**

```
                 Plot of resid11*pred11.  Symbol used is '*'.

resid11 ,
        ,
    1.5 ˆ
        ,
        ,
        ,
        ,
        ,
        ,
    1.0 ˆ                *
        ,          *
        ,
        ,
        ,
        ,                                      *
        ,
    0.5 ˆ
        ,
        ,
        ,                                                   * *
        ,                   **
        ,              *       *                        *       *
        ,                                                   *   *
    0.0 ˆ                                                    *
        ,          *     *  *                                **
        ,              *   *                            *    *
        ,                  *
        ,                      *                         *
        ,                                           *
        ,                    *                      *
   -0.5 ˆ      *
        ,
        ,                                                *
        ,
        ,
        ,      *
        ,
   -1.0 ˆ
        ,
        Šƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒ
        94.5         95.0         95.5         96.0         96.5         97.0

                                        pred11

NOTE: 4 obs hidden.
```

Appendix 8.7. Residual plot for **DM milled bush feed samples**

```
                 Plot of resid12*pred12.  Symbol used is '*'.

resid12 ,
        ,
   1.00 ˆ
        ,
        ,
        ,                        *
   0.75 ˆ
        ,                            *
        ,                            *
        ,                                    *     *
   0.50 ˆ
        ,
        ,
        ,
   0.25 ˆ                                 *
        ,                                      *
        ,              *                      **
        ,            *    *
   0.00 ˆ                                        *
        ,              *                      *  * *
        ,            *
        ,       *    *
  -0.25 ˆ       *
        ,
        ,
        ,
  -0.50 ˆ           *    *                                    *
        ,
        ,           *    *
        ,
  -0.75 ˆ                                                     *
        ,                                                 *
        ,                                                 *
        ,
  -1.00 ˆ
        ,
        Šƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒ
         91           92           93           94           95           96

                                        pred12

NOTE: 7 obs hidden.
```

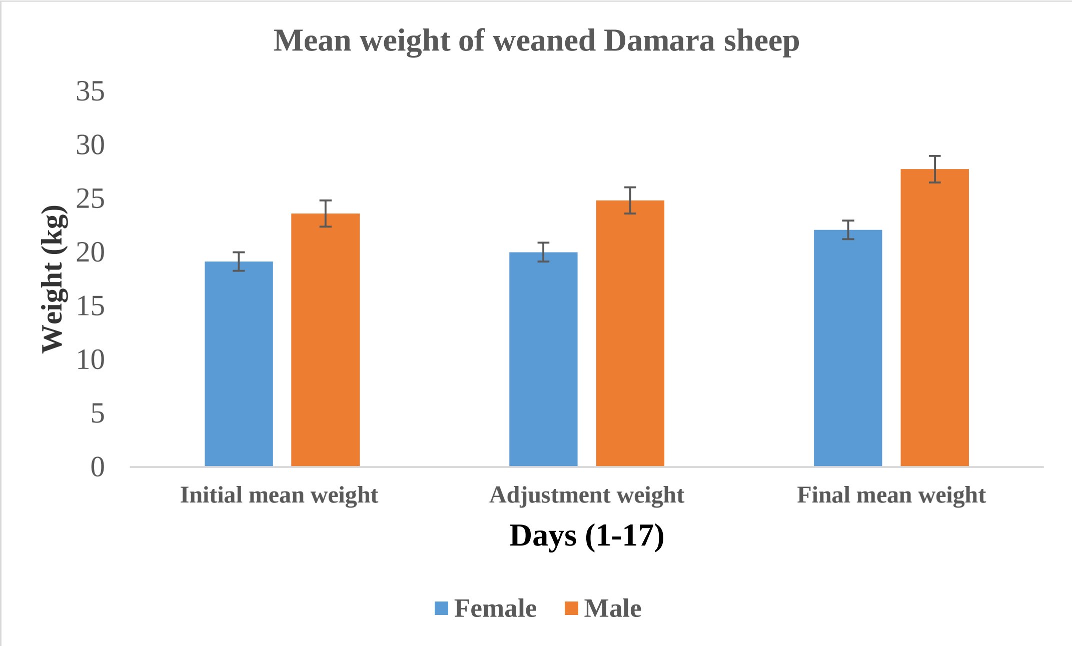
Appendix 8.8. Residual plot for **Ash milled bush feed samples**

```
                 Plot of resid13*pred13.  Symbol used is '*'.

resid13 ,
        ,
    1.0 ˆ
        ,
        ,                                                         *
        ,
        ,             *
        ,
        ,
    0.5 ˆ                                                         *
        ,                   *                      *
        ,                   *
        ,                                        *
        ,             *                             *    *
        ,         **    *                           *  *    *
        ,          **                               *
    0.0 ˆ       *   *
        ,       *
        ,               *                        *       *
        ,                                          **
        ,           *
        ,         *
        ,
   -0.5 ˆ
        ,
        ,
        ,                         *
        ,
        ,
        ,                                                   *
   -1.0 ˆ
        ,                                              *
        ,
        ,
        ,
        ,
        ,
   -1.5 ˆ
        ,
        Šfˆffffffffffffˆffffffffffffˆffffffffffffˆffffffffffffˆffffffffffffˆff
         3.5          4.0          4.5          5.0          5.5          6.0

                                        pred13

NOTE: 4 obs hidden.
```

Appendix 8.10. Residual plot for **CP milled bush feed samples**

```
                 Plot of resid15*pred15.  Symbol used is '*'.

resid15 ,
        ,
   0.04 ˆ                  *
        ,
        ,
        ,
        ,
        ,                   *
        ,
   0.02 ˆ                   *                                      *
        ,
        ,                                                      *
        ,                                                      **
        ,                                                        *
        ,                                                581*
        ,               *                                *      *
   0.00 ˆ               *  *                                  *
        ,          *                                          *
        ,
        ,          *     ** *
        ,                  **
        ,                 *
        ,                *   *
  -0.02 ˆ
        ,                    *                                    *
        ,                                                         *
        ,
        ,
        ,
        ,
  -0.04 ˆ                                                       *
        ,
        Šƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒƒƒƒƒƒƒƒƒƒƒˆƒƒ
        0.20         0.25         0.30         0.35         0.40         0.45

                                        pred15

NOTE: 6 obs hidden
```

Appendix 8.11. Mean weight for weaned Damara sheep at days 1, 5 and 17 of the palatability experiment



Appendix 8.12. Damara sheep consuming *Senegalia mellifera* bush-based feed during a palatability trial.

Appendix 8.13. Ethical clearance certificate

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ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: NEUC /477/2018 **Date: 24 July, 2019**

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: Effects of Chemical treatment On Fibre Content, Digestibility and Palatability of *Senegalia mellifera* and *Catophractes alexandrii* Bush-based Feeds

Student: HOTSTICKS NDOZI

Student Number: 201084767

Campus: Neudamm Campus
Supervisors: *Dr. E. Lutaaya (Main) Prof. J. Mupangwa (Co)*

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.

UREC wishes you the best in your research.

Dr. M. Janik Chairperson

Ms. P. Claassen: Ethics Coordinator